Molecular Biology of the 3{beta}-Hydroxysteroid Dehydrogenase/{Delta}5-{Delta}4 Isomerase Gene Family

Jacques Simard, Marie-Louise Ricketts, Sébastien Gingras, Penny Soucy, F. Alex Feltus and Michael H. Melner

Endocr. Rev. 2005 26:525-582 originally published online Jan 4, 2005; , doi: 10.1210/er.2002-0050

To subscribe to Endocrine Reviews or any of the other journals published by The Endocrine Society please go to: http://edrv.endojournals.org//subscriptions/
Molecular Biology of the 3β-Hydroxysteroid Dehydrogenase/Δ<sup>5</sup>-Δ<sup>4</sup> Isomerase Gene Family

Jacques Simard, Marie-Louise Ricketts, Sébastien Gingras, Penny Soucy, F. Alex Feltus, and Michael H. Melner

Canada Research Chair in Oncogenetics (J.S., M.-L.R., S.G., P.S.), Oncology and Molecular Endocrinology Research Center, Laval University Medical Center, and Laval University, Quebec, Canada G1V 4G2; and Departments of Obstetrics/Gynecology and Cell Biology (F.A.F., M.H.M.), Vanderbilt University School of Medicine, Nashville, Tennessee 37232

The 3β-hydroxysteroid dehydrogenase/Δ<sup>5</sup>-Δ<sup>4</sup> isomerase (3β-HSD) isoenzymes are responsible for the oxidation and isomerization of Δ<sup>5</sup>-3β-hydroxysteroid precursors into Δ<sup>4</sup>-ketosteroids, thus catalyzing an essential step in the formation of all classes of active steroid hormones. In humans, expression of the type I isoenzyme accounts for the 3β-HSD activity found in placenta and peripheral tissues, whereas the type II 3β-HSD isoenzyme is predominantly expressed in the adrenal gland, ovary, and testis, and its deficiency is responsible for a rare form of congenital adrenal hyperplasia. Phylogeny analyses of the 3β-HSD gene family strongly suggest that the need for different 3β-HSD genes occurred very late in mammals, with subsequent evolution in a similar manner in other lineages. Therefore, to a large extent, the 3β-HSD gene family should have evolved to facilitate differential patterns of tissue- and cell-specific expression and regulation involving multiple signal transduction pathways, which are activated by several growth factors, steroids, and cytokines. Recent studies indicate that HSD3B2 gene regulation involves the orphan nuclear receptors steroidogenic factor-1 and dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome gene 1 (DAX-1). Other findings suggest a potential regulatory role for STAT5 and STAT6 in transcriptional activation of HSD3B2 promoter. It was shown that epidermal growth factor (EGF) requires intact STAT5; on the other hand IL-4 induces HSD3B1 gene expression, along with IL-13, through STAT 6 activation. However, evidence suggests that multiple signal transduction pathways are involved in IL-4 mediated HSD3B1 gene expression. Indeed, a better understanding of the transcriptional factors responsible for the fine control of 3β-HSD gene expression may provide insight into mechanisms involved in the functional cooperation between STATs and nuclear receptors as well as their potential interaction with other signaling transduction pathways such as GATA proteins. Finally, the elucidation of the molecular basis of 3β-HSD deficiency has highlighted the fact that mutations in the HSD3B2 gene can result in a wide spectrum of molecular repercussions, which are associated with the different phenotypic manifestations of classical 3β-HSD deficiency and also provide valuable information concerning the structure-function relationships of the 3β-HSD superfamily. Furthermore, several recent studies using type I and type II purified enzymes have elegantly further characterized structure-function relationships responsible for kinetic differences and coenzyme specificity. (Endocrine Reviews 26: 525–582, 2005)
The third option is that, after 17α-hydroxylation and proceed down the pathway to produce the mineralocorticoid, aldosterone. Second, it may undergo 17α-hydroxylation and proceed down the C21,17-diketo pathway to form the principal glucocorticoid, cortisol.

chorionic gonadotropin; 3

various types of human enzymes catalyzing 3

diols; 3

reductase; 17α-PREG, 17α-hydroxyprogrenolone (17OH-PREG), DHEA, and androst-5-ene-3β,17β-diol (Δ5-DIONE) into their respective Δ5-steroids, namely progesterone (PROG), 17α-hydroxyprogesterone (17OH-PROG), Δ4-DIONE, and T. Therefore, this bifunctional dimeric enzyme is required for the biosynthesis of all classes of steroid hormones, namely glucocorticoids, mineralocorticoids, PROG, androgens, and estrogens (Fig. 1). In addition, enzymes of the 3β-HSD family also catalyze the formation and/or degradation of 5α-androstanes and 5α-pregnanes, such as dihydrotestosterone (DHT) and dihydroprogesterone (DHP) (8–10). The 3β-HSD isoenzyme therefore controls critical steroidogenic reactions in the adrenal cortex, gonads, placenta, and a variety of peripheral target tissues (24).

Transient expression of human 3β-HSD isoenzymes provided the first direct evidence that the 3β- and Δ5,4-isomerase activities reside within a single protein (25–27). However, data obtained from affinity alkylation (28) and inhibition experiments (29) that suggested separate 3β- and Δ5,4-HSD activity (23).

A. Subcellular localization

Many of the enzymes of the steroidogenic pathway are localized to the smooth ER with the notable exceptions of P450scc (P450 cholesterol side-chain cleavage; CYP11A1), P450c11 (CYP11B1), and aldosterone synthase (CYP11B2). 3β-HSD subcellular localization patterns are unique in that they show various degrees of ER and mitochondrial distribution. The relevance of dual localization is unclear, yet it can be hypothesized that substrate accessibility could be limited with higher degrees of mitochondrial expression due to reduced mitochondrial transport. This would be analogous to the endocrine community.

Endocrine Reviews is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
the inability of high-efficiency catalysis of cholesterol by P450sc in the absence of the protein controlling cholesterol shuttling, steroidogenic acute regulatory protein (StAR) (34). Smooth ER localized 3β-HSD presumably would have a greater access to cytosolic steroid precursors, such as DHEA and Δ5-DIOL.

3β-HSD activity was detected using histochemical techniques as early as 1965 (35, 36). Similar techniques isolated this activity to the smooth ER and mitochondrial cristae (37). Its membrane localization was not known until studies localized 3β-HSD to the microsomal fraction of human adrenal (38) and chorion/amnion fetal membranes (39), suggesting that 3β-HSD is a membrane-associated enzyme. With the development of antibodies against 3β-HSD, the resolution of its localization increased, and it was verified that it is associated with the ER and mitochondria in human placenta (18, 40, 41), bovine adrenocortical cells (20), and rat adrenal tissue (21).

Submitochondrial fractionation studies show that bovine adrenal 3β-HSD is associated with the inner membrane and with a particulate fraction characterized by contact sites between the two membranes. 3β-HSD activity was higher in this fraction than in the inner mitochondrial membrane, suggesting that intermembrane contact sites may facilitate both the access of cholesterol to the inner membrane where P450sc is localized and the rapid conversion of PREG to PROG by 3β-HSD (19). Elegant biochemical studies have confirmed that a significant amount of adrenocortical 3β-HSD is present in the inner mitochondrial membrane (42). Coprecipitation studies have shown that 3β-HSD is in a functional steroidogenic complex with P450sc in the inner mitochondrial membrane (43), which provides the enzyme

Fig. 1. Schematic representation of the major mammalian steroidogenic pathways. All P450s are cytochrome enzymes. P450c18, This enzyme mediates 11β-hydroxylation and subsequent reactions involved in the biosynthesis of aldosterone; P450c11, 11β-hydroxylase; P450aro, P450 aromatase.
with immediate substrate metabolized from cholesterol transported across the mitochondrial membrane. Other work has shown that subcellular distribution in bovine and murine adrenal tissues demonstrated a higher degree of microsomal to mitochondrial localization (44, 45). A similar subcellular distribution was also recently reported in rat ovary, as revealed by immunoelectron microscopic localization, whereas in the testis, the 3β-HSD was restricted to the mitochondria (45).

Although the functional significance of differential 3β-HSD subcellular localization is unknown, studies have been performed to determine whether the dynamics of 3β-HSD subcellular localization can be altered by regulation. Because Ca2+ flux mediates K+ and A-II increases in aldosterone production by zona glomerulosa (ZG) (46), it is possible that Ca2+ could affect the mitochondrial to ER ratio of 3β-HSD. However, bovine ZG cells showed that neither Ca2+ nor A-II had any effect on the subcellular distribution of 3β-HSD and P450scc, but it did affect STAR localization (22). Another report showed that microsomal 3β-HSD activity in the ovary was unchanged during mouse estrous, yet mitochondrial 3β-HSD activity increased and doubled during diestrus in the mouse (47). These results suggest that 3β-HSD activity could be preferentially distributed to the mitochondria under certain physiological conditions, but this may not be a general phenomenon.

II. Human Type I and II 3β-HSD Genes and Pseudogenes

During the past decade, the structure of the isoenzymes of the 3β-HSD family has been characterized in the human and several other vertebrate species (Fig. 2). Human type I 3β-HSD cDNA was isolated and characterized by Luu-The et al. (18, 48, 49) after purification of the 3β-HSD enzyme from human placenta, and this sequence was later confirmed by other workers (25, 50). The second human 3β-HSD isoenzyme, chronologically designated as type II, was isolated from a human adrenal cDNA library (27). The type I 3β-HSD gene (HSD3B1) encodes an enzyme of 372 amino acids predominantly expressed in the placenta and peripheral tissues, such as the skin (principally in sebaceous glands), mammary gland, prostate, and several other normal and tumor tissues (27, 51–54). The purified enzyme has a Michaelis constant (Km) of 3.7 μM and maximal velocity (Vmax) of 43 nmol/min·mg for 3β-HSD substrate (DHEA) and a Km of 28 μM and Vmax of 598 nmol/min·mg for the isomerase substrate (5-androstene-3,17-dione) (54). In comparison, the type II gene (HSD3B2), which encodes a protein of 371 amino acids, shares 93.5% identity with the type I and is almost exclusively expressed in the adrenals, the ovary, and testis (27, 54, 55). The purified enzyme has a Km of 47 μM and Vmax of 82 nmol/min·mg for 3β-HSD substrate (DHEA) and a Km of 88 μM and Vmax of 970 nmol/min·mg for the isomerase substrate (5-androstene-3,17-dione). The higher affinity of type I 3β-HSD could facilitate steroid formation from relatively low concentrations of substrates usually present in peripheral tissues. Based on their differential tissue-specific expression pattern, it is not surprising that classical 3β-HSD deficiency, which will be discussed further in Section VII, results from mutations in the HSD3B1 gene, whereas the HSD3B1 gene is normal in affected individuals (56–59).

The structure of each of the HSD3B1 and HSD3B2 genes consists of four exons which are included within a DNA fragment of 7.8 kb and which share 77.4, 91.8, 94.5, and 91.0% identity, respectively (26, 60, 61). The genes are assigned to chromosome 1p13.1, 1–2 cM from the centromeric marker D1Z5 (Fig. 3) (62, 63). Our initial data suggested that the HSD3B1 and HSD3B2 genes and three related pseudogenes (64) are included within a 0.29 megabase SacII DNA fragment, suggesting that the human 3β-HSD gene family exists as a tandem cluster of related genes (63) as observed for the mouse β-HSD genes (65). In support of these findings, in addition to the two expressed genes in the human, five pseudogenes have also been recently cloned and physically mapped (66) (Fig. 3). HSD3B1ψ1–5 are unprocessed pseudogenes that are closely related to HSD3B1 and HSD3B2 genes, but contain no corresponding open reading frames. Although mRNA is expressed from ψ4 and ψ5 in several tissues, altered splice sites disrupt the reading frames. The two expressed genes, HSD3B1 and HSD3B2, are located in direct repeat, 100 kb apart; however, separation by two pseudogenes, ψ1 and ψ2, prevents them from sharing common promoter elements (66).

III. Structure-Function Relationships

The two-step reaction of the 3β-HSD/isomerase involves the reduction of NAD+ to NADH by the rate-limiting 3β-HSD activity and the requirement of this NADH for the activation of the isomerase on the same enzyme (41, 67). Stopped-flow spectroscopy studies show that NADH activates the isomerase activity by inducing a time-dependant conformational change in the enzyme, suggesting that the 3β-HSD and isomerase domains of the enzyme are linked by a shared coenzyme domain that functions both as the binding site for NAD+ during the 3β-HSD reaction and as the coenzyme domain for the allosteric activation of the isomerase reaction (33).

The 3β-HSD isoenzymes belong to the short-chain alcohol dehydrogenase superfamily, mainly determined by the nucleotide-binding site sequence located at the amino terminus. It consists of a β-strand, α-helix, β-strand in a fold that provides a hydrophobic pocket for the AMP part of the nucleotide factor. The turn between the first β-strand and the α-helix is a glycine-rich segment, Gly-X-X-Gly-X-Gly, similar to the common Rossmann fold sequence Gly-X-Gly-X-
X-Gly conserved among most NAD(H)-binding enzymes (68). This well-conserved glycine-rich fragment forms a hydrophobic pocket that allows close association of the AMP part of the cofactor. A preliminary study of rat type III enzyme has targeted Asp (36) as the amino acid that may be responsible for the strict NAD$^+$ specificity of the enzyme (8).

More recent mutagenesis studies in human type I enzyme demonstrate that the D36A/K37R mutant shifts cofactor preferences of both 3$\beta$-HSD and isomerase activities from NAD(H) to NADP(H), thus showing that the two activities utilize a common coenzyme domain (69).

Affinity labeling of purified human type I identified two tryptic peptides, comprising amino acids Asn176 to Arg186 and Gly251 to Lys274 that contain residues involved in the putative substrate-binding domain (30). These studies have shown that the Gly251 to Lys274 peptide was associated with the site of isomerase activity, whereas Tyr253 appears to function as the general proton donor in the isomerase reaction (24). His261 also appears to be a critical residue for the 3$\beta$-HSD activity (23). Additional kinetic analyses of D257L and D258L mutants suggest that this region is part of the isomerase substrate domain (69).

In contrast to other short chain dehydrogenases with a single catalytic Y-X-X-X-Y motif (5, 70, 71), there are two potential catalytic motifs (Y$^{154}$-X-X-X-K$^{158}$ and Y$^{269}$-X-X-X-K$^{273}$) in the primary structure of all 3$\beta$-HSDs. Human type I and type II only differ at position 156 in this motif, type I having a tyrosine whereas type II has a histidine residue. The H156Y mutant form of the type I enzyme shifts the substrate kinetics for DHEA and PREG to the same Km and Vmax values exhibited by the type II enzyme; thus, H$^+$ in the type I vs. Y$^{156}$ in type II 3$\beta$-HSD accounts for the substantially higher affinity of the type I 3$\beta$-HSD activity for these substrates and inhibitor epostane relative to the type II enzyme (72).

Two membrane-binding domains lying between residues 72 and 89 in the NH$_2$-terminal region and between residues 283 and 310 in the COOH-terminal region were identified. Indeed, deletion of the 283–310 region causes the enzyme to localize in the cytosol without affecting its activities (73). The region is therefore a critical membrane domain of 3$\beta$-HSD that can be deleted without compromising enzyme function (54, 73). Deletion of residues 72–89 in the NH$_2$-terminal region produces a mutant protein that is distributed among the microsomes, mitochondria, and cytosol (73). Because 3$\beta$-HSD and isomerase activities remain in the membranes of microsomes and mitochondria, the presence of the 283–310 domain in this mutant allows the protein to retain significant hydrophobicity. However, a majority (72%) of the protein is shifted into the cytosol, so the 72–89 region does contribute to membrane association. The 8-fold loss of both 3$\beta$-HSD and isomerase activity that results from the 72–89 deletion underscores the importance of this region to enzyme function (73). The data obtained by Thomas’ group (73) with the human type I enzyme are consistent with one of our previous studies in which the increased polarity of the domain between residues 75 and 91 in the rat type II 3$\beta$-HSD/isomerase was responsible for its having much lower activity than the rat type I enzyme (74). Thus, the presence of this highly conserved hydrophobic domain may be crucial to activity in the entire 3$\beta$-HSD gene family. The expression of an active soluble 283–310 deletion mutant of the type I enzyme in a baculovirus expression system provides a valuable tool for crystallographic studies that may ultimately determine the tertiary/quaternary structure of the enzyme (73). A
three-dimensional ribbon model has been constructed by Thomas’ group (69), based on the homology data for human type I 3β-HSD and UDP-galactose-4-epimerase. This also represents a useful tool for interpreting biochemical data and designing inhibitors (Fig. 4) (69).

IV. Evolution of the 3β-HSD Gene Family

Multiple 3β-HSD isoenzymes have been cloned from several other species, further illustrating that the 3β-HSD gene family is conserved in vertebrate species (Fig. 2 and Table 1). The tissue-specific expression of multiple members of the 3β-HSD family was first demonstrated in the rat (75). Other 3β-HSD cDNAs have been cloned using adrenal/gonadal cDNA libraries from six other species, namely the macaque ovary (76), bovine ovary (77), chicken adrenal (78), horse testis (79), rainbow trout ovary (80), and eel ovary (81). It is important to note that in contrast to the human, which is designated as type II, the adrenal/gonadal 3β-HSD isoenzymes in all other vertebrate species have been designated as type I, due to the chronological order in which they were cloned. The only 3β-HSD sequence available from the pig was obtained using a cDNA library from adipose tissue (616).

A. The rat 3β-HSD gene family

The secretion of sex steroids originates exclusively from the gonads in rodents and domestic animals, which is in contrast to humans who in addition to secreting sex steroids from the gonads, secrete the sex precursors DHEA and DHEA-S from the adrenal gland. The structures of four members of the rat 3β-HSD family have been characterized (75, 82, 83). With the exception of type III, all isoenzymes catalyze the transformation of 5-pregnen-3β-ol and 5-androsten-3β-ol steroids into the corresponding Δ4-3-ketosteroids as well as the interconversion of 3β-hydroxy- and 3-keto-5α-androstanone steroids. The various isoenzymes show differences in tissue-specific expression (84) (Fig. 5). The rat type I and II 3β-HSD proteins are expressed in the adrenals, gonads, kidney, placenta, adipose tissue, and uterus and share 93.8% identity. The type III protein shares 80% identity with the type I and II proteins but, in contrast to other types, is a specific 3-KSR. The type III gene is exclusively expressed in the kidney, placenta, adipose tissue, and uterus and share 93.8% identity. The type III protein shares 80% identity with the type I and II proteins but, in contrast to other types, is a specific 3-KSR. The type III gene is exclusively expressed in

Table 1. Kinetic parameters and major expression sites of 3β-HSDs from human, rat, mouse, hamster, macaque, bovine, and rainbow trout

<table>
<thead>
<tr>
<th>Species</th>
<th>Type</th>
<th>Km PREG/DHEA (μM)</th>
<th>Cofactor</th>
<th>Major sites of expression</th>
<th>First cloning (Ref. no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>I</td>
<td>&lt;1</td>
<td>NAD⁺</td>
<td>Placenta, skin and mammary gland</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1 to 4</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>27</td>
</tr>
<tr>
<td>Rat</td>
<td>I</td>
<td>&lt;1</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>&gt;10</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3-KSR</td>
<td>NADPH</td>
<td>Male liver</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>&lt;1</td>
<td>NAD⁺</td>
<td>Placenta and skin</td>
<td>83</td>
</tr>
<tr>
<td>Mouse</td>
<td>I</td>
<td>&lt;1</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>nd</td>
<td>nd</td>
<td>Kidney and liver</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>&lt;1</td>
<td>NAD⁺</td>
<td>Liver &gt; kidney</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>3-KSR</td>
<td>NADPH</td>
<td>Kidney</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>3-KSR</td>
<td>NADPH</td>
<td>Male liver</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td>&lt;1</td>
<td>NAD⁺</td>
<td>Placenta and skin</td>
<td>99</td>
</tr>
<tr>
<td>Hamster</td>
<td>I</td>
<td>2 to 5.5</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2 to 9</td>
<td>NAD⁺</td>
<td>Kidney and liver</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>3-KSR</td>
<td>NADPH</td>
<td>Male liver</td>
<td>96</td>
</tr>
<tr>
<td>Macaque</td>
<td>nd</td>
<td>nd</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>76</td>
</tr>
<tr>
<td>Bovine</td>
<td>&gt;10</td>
<td>NAD⁺</td>
<td>Adrenals and gonads</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>DHEA &gt; PREG</td>
<td>NAD⁺</td>
<td>Ovary</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

nd, Not determined.
male liver, and there is marked sexual dimorphic expression, which results from pituitary hormone-induced gene repression in the female rat liver (75, 85). The rat type IV protein shares 90.9, 87.9, and 78.8% identity with that of types I, II, and III proteins, respectively, and is the prominent mRNA species detectable in the placenta and the skin (83). In this respect, it is therefore possible that the rat type IV and the human type I proteins have conserved cis-acting elements in their promoter regions, involved in tissue-specific transcriptional control common to skin and placenta (8). The activities of rat types I and IV are similar (83), whereas there is much lower enzyme activity for the type II compared with the type I, which could be due to a change in four amino acid residues located in a putative membrane-spanning domain, between residues 75 and 91 as described in the previous section (74). Furthermore, types I and IV possess a 17α-HSD activity specific to 5α-androstane-17β-ol steroids, thus suggesting a key role in controlling the bioavailability of the active androgen DHT (84, 86).

B. The mouse 3β-HSD gene family

To date, six distinct cDNAs encoding murine members of the 3β-HSD family have been cloned (87–92), all of which are highly homologous and encode a protein of 372 amino acids. The murine family of 3β-HSD enzymes has been extensively reviewed in the literature (9, 93) and references therein. The genes encoding the different isoenzymes are found closely linked on mouse chromosome 3 (65). Hybridization by Southern blot analysis of restriction enzyme-digested yeast artificial chromosome DNA using an 18-base oligonucleotide that hybridizes without mismatch to all known Hsd3b sequences indicates that there are a total of seven Hsd3b genes or pseudogenes in the mouse genome. Additional analysis of mouse genomic DNA by pulse field gel electrophoresis suggests that all of the Hsd3b gene family is found within a 400-kb fragment (9, 94). The different forms are expressed in a tissue-specific and developmentally specific manner and fall into two functionally distinct classes of enzymes (92). 3β-HSD types I and III, and most probably type II, function as dehydrogenase/isomerases, and are essential for the biosynthesis of active steroid hormones, whereas 3β-HSD type IV and type V, analogous to rat type III, function as 3-KSRs and are therefore involved in the inactivation of active steroid hormones (88, 91). 3β-HSD I in the adult mouse is expressed in the gonads and the adrenal gland (87), whereas 3β-HSD II and III are expressed in the liver and kidney (87), with much greater expression of type III in the liver than in the kidney. The major site of expression of 3β-HSD IV is in the proximal tubules of the kidney in both the male and female mice (89), with minor expression in the testis (91).
type V isoenzyme appears to be expressed only in the liver of the male mouse, with expression starting during the latter half of pubertal development (91, 95). The type VI isoenzyme functions as a NAD-dependent 3β-HSD and is the earliest isof orm to be expressed during the first half of pregnancy in cells of embryonic origin and in uterine tissue (92). In the adult mouse, 3β-HSD type VI appears to be the only isoenzyme expressed in the skin and is also expressed in Leydig cells of the testis, although to a lesser extent than type I 3β-HSD.

It is hypothesized that mouse 3β-HSD type VI cDNA is orthologous to human 3β-HSD type I cDNA, which has been shown to be the only isoenzyme expressed in the placenta and the skin. The demonstration that the type VI isoenzyme in the mouse functions as a 3β-HSD and is the predominant isoenzyme expressed during the first half of pregnancy in uterine tissue and embryonic cells, suggests that this isoenzyme may be involved in the local production of PROG, which is required for the successful implantation of the blastocyst and/or maintenance of pregnancy (92).

C. The hamster 3β-HSD gene family

The hamster is a rodent species, but in contrast to the rat and mouse, in which the principal corticosteroid is corticosterone, the principal corticosteroid in the hamster is cortisol. A study on the regulation of adrenal steroidogenic enzymes suggested that the hamster could be a good model for studying human steroidogenesis. With this in mind, three isoenzymes of 3β-HSD were characterized in the hamster (96, 97). The type I isoenzyme was isolated from an adrenal cDNA library and was identified as being a low Km 3β-HSD (Km: PREG, 5.5 μm; DHEA, 2.4 μm). A separate isozyme, designated type II was isolated from the kidney and was also found to be a low Km 3β-HSD (Km: PREG, 8.8 μm; DHEA, 2.9 μm). Two cDNAs were isolated from the liver, one which was identical to the type II sequence found in the kidney, and a distinct cDNA encoding an isoform designated as type III, which does not possess any steroid dehydrogenase activity but functions as a 3-KSR. There is sexual dimorphic expression of this liver-specific type III 3β-HSD in the hamster, as seen for the rat liver-specific type III KSR. As is the case for both the rat and mouse, a high affinity 3β-HSD is expressed in the adrenal and gonad of the hamster, consistent with the steroidogenic role of these tissues (96).

D. Phylogeny of the 3β-HSD gene family

McBride et al. (66) indicated no evidence for the presence of other members of the human 3β-HSD family within the physical contig of 0.5 Mb by Southern blot analysis, thus suggesting that in humans there is no comparable liver-specific 3-KSR sharing a high percentage of identity with other members of the HSD3B cluster. Such a conclusion is also well supported by phylogenetic analysis of the mammalian 3β-HSD gene family. Unexpectedly, the phylogenetic tree strongly suggests that independent gene duplications occurred in different species (66, 91), (V. Laudet, personal communication). As illustrated in Fig. 6, our recent analysis shows a first complex of three genes from primates and suggests that an ancestral gene duplicated specifically in the...
It is also of interest to note that although the N-terminal amino acid sequences of the pig hepatic 3β-hydroxy-Δ⁵-C₂⁷-steroid dehydrogenase and the vertebrate 3β-HSD enzymes show some similarities, substrate specificities differ. Although vertebrate 3β-HSD/Δ⁵-Δ⁴ isomerase enzymes are active on C₁₉/C₂₁ steroids, porcine hepatic 3β-hydroxy-Δ⁵-C₂⁷-steroid dehydrogenase is active on C₂⁷ steroids such as 7α-hydroxycholesterol, 7α-25-dihydroxycholesterol, 7α-27-dihydrocholesterol, and 3β-7α-dihydroxy-5-cholestenoic acid, and participates in bile acid biosynthesis (98, 99) (Fig. 7). Furthermore, genetic studies of a kindred affected with 3β-hydroxy-Δ⁵-C₂⁷-steroid dehydrogenase deficiency, which is associated with hepatic failure in childhood, showed no genetic linkage to the HSD3B cluster (100). In fact, such hepatic and extrahepatic activity was practically unaffected by trilostane, a well-known C₁₉/C₂₁ 3β-HSD inhibitor (99). Gene structure of HSD3B7, as well as positioning of disease-associated mutations on corresponding nucleic and amino acid sequences, is represented in Fig. 8. Moreover, it has recently been suggested that the alcohol dehydrogenase γ-isoenzyme is the sole 3β-HSD using bile acids as a substrate in human liver cytosol (101). Also, it has been demonstrated that the X-linked dominant male-lethal phenotype gene mutated in bare patches and striated mice encodes a novel 3β-HSD (102). This gene encodes an NADPH enzyme, which is likely to be involved in cholesterol biosynthesis and shares only 30% identity with other mammalian 3β-HSD enzymes, thus supporting the phylogenetic divergence between the C₁₉/C₂₁ 3β-HSD/Δ⁵-Δ⁴ isomerase and the other enzymes involved in bile acid metabolism and/or biosynthesis of cholesterol.

E. Enzymatic characteristics of the 3-KSRs (rat liver-specific type III, mouse types IV and V, and hamster type III)

As mentioned briefly above, the rat type III protein (75) does not display oxidative activity for the classical substrates PREG, DHEA, Δ⁵-DIOL, and 3β-DIOL, but instead is a specific 3-KSR responsible for the conversion of 3-keto saturated...
steroids, such as DHT and DHP, into inactive steroids using NADH phosphate (NADPH) as cofactor instead of NADH (86). In addition to using 5α-androstane steroids such as DHT and androstanedione as substrates, the expressed rat 3-KSR also catalyzes the 3α-reduction of DHP into 5α-pregnane-3α,20β-diol (84). The Km and Vmax values of the expressed 3-KSR protein using DHP as substrate and NADPH as co-factor were calculated to be 0.24 μM and 0.83 nmol/min/mg protein, respectively. In comparison, the Km value of the expressed type I 3α-HSD isoenzyme, also using DHP as the substrate in the presence of NADH as cofactor, was 0.55 μM, whereas the calculated Vmax value was 0.18 nmol/min/mg protein (84).

Examination of the 3α-HSD isoenzymes shows a typical βαβ dinucleotide-binding fold with Asp (36) located in the position predicted for the acidic residue that participates in hydrogen bond formation with the 2'-hydroxyl moiety of the adenosine ribose of all known NAD-dependent dehydrogenases (Fig. 2). Using site-directed mutagenesis, it has been shown that the presence of a Tyr residue instead of an Asp residue at position 36 in the typical βαβ dinucleotide-binding fold of the cofactor binding domain of rat type III is responsible for the difference in cofactor specificity of the rat 3-KSR (type III) protein, but this alteration is not sufficient to explain the low activity of the enzyme with Δ5-3β-hydroxysteroid substrates (103). The physiological importance of this peculiar member of the rat 3α-HSD family is well supported by the finding that mouse types IV and V and hamster type III also possess this specific 3-KSR activity (88, 91, 96). The 3-KSR activity of these three latter enzymes is most likely due to the presence of Phe (36) in place of Arg (36), as suggested by the data resulting from the study on Asp (36) in the rat type III sequence.

**F. 17β-HSD activity of rat type I and IV 3β-HSDs**

Using cell homogenate preparations, it was first noted that the rat type I 3β-HSD protein has androgenic 17β-HSD-like activity...
activity. It was found that the affinity for the DHT substrate by the enzyme is similar to that of the substrates for 3β-HSD activity, although with a much lower velocity (86). However, using intact cells transfected with the rat type I, it was found that the enzyme catalyzed almost exclusively the conversion of DHT into androstanediol via the 17β-HSD-like activity. The intrinsic 17β-HSD activity of rat types I and IV 3β-HSD is specific to 5α-androstane-17β-ol steroids, therefore suggesting that it plays a key role in controlling the bioavailability of the active androgen DHT.

The predominance of this secondary 17β-HSD activity over the primary 3β-HSD activity in intact cells most likely results from the high bioavailability of NAD+ in mammalian cells relative to the low levels of the intracellular pool of NADH. The apparent discrepancy between the oxidoreductase activity of the rat type I and IV proteins, as revealed by the interconversion of DHT and 3β-DIOL using homogenate preparations from mammalian cells expressing the recombinant isoforms, and the lack of significant 3-KSR activity, measured in intact cells in culture expressing the same recombinant enzymes, can also be explained by the low levels of the intracellular pool of NADH relative to the high bioavailability of NAD+ in mammalian cells. In agreement with this explanation, in cultured HeLa, JEG-3, and SW-13 cells expressing rat types I and IV 3β-HSDs, Δ5-hydroxysteroid precursors are efficiently converted to their corresponding Δ4-3-keto steroids, which is well known to require NAD+ as the allosteric cofactor. The high efficiency of conversion of DHT into 3β-DIOL in cultured HeLa cells expressing rat 3-KSR (type III) also argues in favor of the bioavailability of NADPH within the cells (84, 86). It is also of interest that 3α-HSD is known to use NADPH as a cofactor, which is in agreement with the predominant metabolic pathways using NAD+ or NADPH as cofactors postulated to be present in transfected cells in culture. These results also emphasize the important fact that assessment of enzyme activity should always be performed in intact cells to more closely resemble the situation in vivo, an issue that will be discussed in more detail in Section VII.

In relation to an enzyme having dual activity, such secondary activity could be explained by the binding of the steroid in the inverted substrate orientation, in this case from the C-17 rather than C-3 extremity, to the same active site responsible for the primary activity of the enzyme. The physiological relevance of this secondary 17β-HSD activity is also supported by the observation that the purified bovine adrenal 3β-HSD enzyme (20) also possesses the 17β-HSD-like activity (84, 86). Moreover, it was recently shown that mouse types I and VI 3β-HSD isoforms display significant 17β-HSD-like activity (617).

Another enzyme known to catalyze the oxidation of both 3β- and 17β-hydroxy groups of certain hydroxysteroids is the NAD+-dependent 3β-17β-hydroxysteroid dehydrogenase from Pseudomonas testosteronei (104). The elucidation of the cDNA sequence of this enzyme revealed that it is a member of the short-chain dehydrogenase/reductase superfamily, however, sharing more homology with the 17β-HSD than with the 3β-HSD enzymes (105). It is also known that some members of the 17β-HSD family, such as human type 2 (106) and human type 7 (107) 17β-HSDs, possess dual 3β/17β activity.

V. Transcriptional Regulation of Human Type I and II 3β-HSD

The human HSD3B2 gene is the major form expressed in the adrenal cortex, ovary, and testicular Leydig cells. It is most homologous to the type I gene expressed in mice, rats, and other species. The different gene isoforms are so named because of their chronological isolation. The current state of knowledge concerning the transcriptional control human HSD3B2 gene will be discussed first.

A. Gonadal/adrenal isoenzyme—type II 3β-HSD

1. Steroidogenic factor-1 (SF-1). Initial studies investigating the transcriptional regulation of the human HSD3B2 gene primarily focused on the trophic hormones known to regulate expression of other genes, including ACTH in the adrenal cortex, LH/human chorionic gonadotropin (hCG) in theca cells and corpus luteum (CL), and LH in testis Leydig cells. Until fairly recently, data regarding the transcriptional regulation of 3β-HSD was highly limited. After the isolation and sequencing of genomic clones for human type II 3β-HSD, several studies analyzed the promoter and 5′-flanking regions looking for regulatory elements essential for expression and regulation in steroidogenic cells. Initial examinations of the sequence were not very revealing. Because cAMP was a known intracellular mediator of trophic hormone stimulation of 3β-HSD expression, it was interesting that no identifiable cAMP response elements were observed in the proximal 1.3 kb of the 5′-flanking sequence. There were two putative activator protein-1 (AP-1) elements at −576 and −977 that matched functional AP-1 elements in other genes (e.g., the sequence at −977 is an exact match of an AP-1 element in the simian virus 40 promoter). This limited information formed the basis of the initial functional studies of the 3β-HSD promoter by transfection into steroidogenic cells.

The H295R human adrenocortical carcinoma cell line was chosen as a steroidogenic cell model due to its relatively high level of differentiated functions, including responsiveness to adrenal trophic hormones such as ACTH and synthesis of cortisol. Interestingly, these cells have characteristics of all three zones of the normal adrenal gland, the zona reticularis (ZR), zona fasciculata (ZF), and ZG (108). As such, these cells may represent a pluripotent adrenal cortical stem cell that transformed before terminal differentiation. The H295R cells can be cultured under different specific conditions that promote the differentiated characteristics of cells from the individual zones (108). In these studies, the cells were treated with activators of the protein kinase A (PKA) pathway, such as forskolin and dibutyryl cAMP, to increase the expression of enzymes P450 17α-hydroxylase/17,20-lyase (P450c17), 3β-HSD, and P450c11 as well as cortisol production, thereby promoting the characteristics of ZF cells. Promoter-reporter constructs were prepared that used a series of 5′-deletions of the human HSD3B2 5′-flanking sequence and promoter, fused to the chloramphenicol acetyltransferase (CAT) gene. Transfections were performed, and the cells were treated in
the presence and absence of phorbol ester stimulation. The surprising finding of these initial studies was that the putative AP-1 elements were nonfunctional with respect to either cAMP or phorbol ester stimulation. Both putative AP-1 elements could be removed without affecting the cAMP- or phorbol ester-stimulated promoter activity, with 5’-deletion down to −100 bp. The promoter-reporter constructs did not lose responsiveness to cAMP or phorbol ester stimulation until a further deletion was made to −52 (109).

The sequence between −100 and −52 contained an element at −64 to −56 that is an 8/9 match for the consensus regulatory element that binds the orphan nuclear receptor SF-1, also referred to as adrenal 4 binding protein. New nomenclature for members of the nuclear receptor superfamily designates this family as NR5A and SF-1 as NR5A1a (110). The essential nature of the SF-1 element was tested further using two approaches. First, promoter-reporter constructs were transfected into a nonsteroidogenic cell type that does not express SF-1 (HeLa cells) in the presence and absence of cotransfection with an SF-1 expression vector. Second, the importance of SF-1 was tested with the use of a 2-bp point mutation in the element (from TCAAGGTAA to TCAATTAA), whereas all other sequences in the −100-bp HSD3B2 gene promoter remained as in the wild type. The 2-bp point mutation in the SF-1 element dramatically abrogated the cAMP and phorbol ester response of the promoter (109). These studies indicated that SF-1 was essential for cAMP or phorbol ester-stimulated steroidogenesis and suggested that trophic hormone stimulation of type II 3β-HSD expression involved SF-1 activation.

The mechanisms by which cAMP and phorbol esters stimulate SF-1-mediated transcription of the HSD3B2 gene are not yet clear. In the GnRH promoter, another SF-1 responsive gene, the essential response of SF-1 involves an interaction between SF-1 and another transcription factor, early growth response protein (111, 112). However, the HSD3B2 promoter differs from the GnRH promoter in that it lacks proximal early growth response recognition sequences. However, it is possible that the interaction of SF-1 with another yet undescribed transcription factor is a necessary component to the cAMP/phorbol ester stimulation.

2. Stat5. The involvement of transcription factors other than SF-1 in the control of the human HSD3B2 gene has become evident following the discovery of a regulatory element, which interacts with Stat5. The Stat family of proteins is named for the acronym (signal transducers and activators of transcription). The Stats comprise a family of cytoplasmic transcription factors that are activated by tyrosine kinases followed by nuclear translocation and binding to specific regulatory elements (reviewed in Ref. 113). The Stats are activated by a number of extracellular protein ligands including cytokines, growth factors, and prolactin (PRL)/GH. The activation involves either tyrosine kinase activity that is intrinsic to the receptors for these ligands or tyrosine kinases that associate with the receptors such as the Janus kinases. Seven different Stat genes have been identified (Stats 1, 2, 3, 4, 5a, 5b, and 6). Targeted gene disruptions have been performed for each of these seven Stat genes in mice, resulting in different phenotypes, which include immune deficiencies in Stat 1 knockouts, embryonic lethals in knockouts of Stats 2 and 3, and deficient breast development and lactation in Stat5 knockouts. Interestingly, the Stat5a Stat5b double knockout displays luteal failure (114), which involves one of the key tissues expressing 3β-HSD.

Stat5 was independently identified and cloned in studies concerning a transcription factor important for PRL activation of the β-casein promoter in mammary epithelial cells (115, 116). The potential involvement of PRL and Stat5 in the human 3β-HSD promoter is an intriguing concept. Prolactin and placental lactogens are known to be important luteotropic hormones in many species including bovine, porcine, and rodent (117). The human placenta expresses human placental lactogen, which was previously referred to as human chorionic somatomammotropin. The involvement of PRL as a potential luteotropic hormone in humans has not been examined in detail, although conditions of hypo- and hyperprolactinemia are known indicators of female infertility (118, 119).

Two independent findings suggested the possibility that Stat5 could be mediating regulation of the 3β-HSD promoter. First, analysis by Western blot showed that Stat5 was induced on d 5 (after hCG) in pseudopregnant rat ovaries approximately 10-fold over controls (120). This was a specific effect because Stat3 levels remained unchanged. These data suggested that Stat5 was being specifically induced during luteinization and that it could be involved in mediating the regulation of luteal function. Because this represents an important site of Stat5-mediated activity, it suggested a role for Stat5 in luteal function. Stat5 is expressed in a wide variety of tissues, and its expression is fairly constitutive. However, it has been shown to be induced in mammary epithelial cells during differentiation of the mammary gland into the active lactation state (121). A second finding was the discovery of a 9/9 match for a Stat5 consensus regulatory element in the human HSD3B2 gene promoter during the examination of SF-1 action. The sequence TTCTGAGAA at −118 to −110 is a 9/9 match to the consensus regulatory element TTC-NNGAA for Stat5 (Fig. 9). These findings suggested a potential regulatory role for Stat5.

Transfection studies have confirmed that PRL activates Stat5 regulation of the human HSD3B2 promoter (122). Point mutations of the Stat5 regulatory element abrogate the PRL response, both the fold-stimulation and the levels of stimulated activity. However, disruption of Stat5 action does not totally remove PRL stimulation. It is possible that some of the alternative signaling pathways stimulated by PRL may contribute to PRL-stimulated 3β-HSD expression.

The functional Stat5 element in the HSD3B2 promoter may also integrate signaling from other ligand-stimulated pathways. For example, angiotensin II has been shown to stimulate Stat5 in cardiac myocytes (123). A similar signaling pathway in adrenal ZG cells would allow angiotensin II stimulation of 3β-HSD expression in the synthesis of mineralocorticoids. Another ligand that could utilize this pathway is EGF. Stat5 has been shown to be the major Stat protein activated in EGF stimulation of the mouse liver (124). Recent studies have demonstrated that EGF stimulates cortisol synthesis in H295 adrenocortical cells as well as stimulating 3β-HSD mRNA levels and promoter activity (125).
more, Stat5 and a functional Stat5 regulatory element in the HSD3B2 promoter are required for this stimulation (Fig. 10) (125).

Stat proteins are important for the transcription of other steroidogenic enzyme genes. For example, Stat3 has been shown to be important for aromatase (CYP19; P450arom) expression in adipocytes (126). The aromatase gene has multiple promoters that are used in tissue-specific expression and regulation (126). Interestingly, the adipocyte promoter activity utilizing the Stat3 element requires glucocorticoids for maximal activity (126). Although the aromatase adipocyte promoter contains a glucocorticoid response element, it is not clear whether additional glucocorticoid effects can occur through interactions between Stat3 and the glucocorticoid receptor.

3. DAX-1. DAX-1 (dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome gene 1) was originally isolated by positional cloning from patients presenting with adrenal congenita hypoplasia associated with hypogonadotropic hypogonadism (127, 128). Mutations in DAX-1 have been found to be the cause of adrenal hypoplasia congenita and hypogonadotropic hypogonadism. The possible role of an atypical member of the nuclear hormone receptor superfamily on 3β-HSD promoter activity has come from studies examining the effects of DAX-1 overexpression on adrenal cell steroidogenesis and steroidogenic enzyme gene expression. The structure of DAX-1 indicates that it lacks the most highly conserved DNA-binding domain typical of members of the nuclear hormone receptor superfamily (127). This has raised questions as to whether this transcription factor binds to DNA. Data have been presented suggesting that DAX-1 has unique mechanisms of DNA binding through putative stem-loop structures (129). An alternative hypothesis is suggested by the sequencing of DAX-1 from another species, which apparently lacks the analogous domain necessary for DNA binding (130). This implies that possible protein-protein interactions between DAX-1 and other transcription factors may be critical to the function of this orphan nuclear receptor. Additional work in this field is needed to determine the mechanisms of DAX-1 actions.

The overexpression of DAX-1 in Y-1 adrenal cells inhibits
There is growing evidence in the literature that steroid hormones modulate type II $\beta$-HSD expression. For example, glucocorticoids stimulate the expression of $\beta$-HSD in adrenal cells (132), whereas androgens inhibit $\beta$-HSD expression in the adrenal cortical cells and in testicular Leydig cells (133, 134). The transcriptional mechanisms by which this regulation is occurring are of high interest because the $\text{HSD3B2}$ gene promoter and 5'-flanking sequence lack clear homologous consensus steroid regulatory elements. One possible explanation for these findings is that the steroid hormones exert their effects using posttranscriptional mechanisms. Alternatively, the steroids could exert their actions indirectly, altering the transcription of another transcription factor, which targets the $\text{HSD3B2}$ gene promoter.

Lastly, another possible mechanism is that the steroid hormones and their cognate nuclear receptors are acting via nonclassical mechanisms to alter transcription. Accumulating evidence indicates that nuclear hormone receptors can alter transcription via protein-protein interactions with other classes of transcription factors and that these interactions do not require direct DNA binding by the receptors (135). Steroid receptors have been shown to interact with Stat proteins (136), AP-1 (137), nuclear factor-$\kappa$B (138), and Sp1 (139) proteins and to alter transcription in a ligand-dependent manner.

It has been recently demonstrated that glucocorticoids stimulate type II $\beta$-HSD mRNA levels in H295R cells and stimulate $\text{HSD3B2}$ gene promoter activity (125). The mechanisms by which this stimulation occurs are beginning to be characterized and are linked to Stat5. Inactivating point mutations in the Stat5 regulatory element of the $\text{HSD3B2}$ gene promoter abolishes glucocorticoid regulation (125). Additionally, point mutations in Stat5, which convert the critical tyrosine phosphorylation residue to a phenylalanine and abolish Stat5 activation, also abolish its regulation by glucocorticoids. These data indicate that Stat5 is critical to the mechanism of $\text{HSD3B2}$ gene promoter activation by glucocorticoids. The exact mechanisms involved in this action are not yet characterized but could involve protein-protein interactions between glucocorticoid receptor and Stat5, as suggested for the $\beta$-casein promoter in mammary glands (136). These data are intriguing because they point to new ways in which nuclear receptors function in transcriptional activation. They also raise a number of questions concerning the established mechanisms of nuclear receptor action. For example, the domains of the nuclear receptor proteins, which are critical for different aspects of their function, have been mapped out in relation to the traditional DNA binding-dependent mechanism of action. With these new nontraditional mechanisms, which domains of the nuclear receptors are critical to their functions? In addition to structure-function questions, what is the influence of known agonists and antagonists on the efficacy of activation? What is the effect of

Fig. 10. EGF activates type II $\beta$-HSD reporter activity through a Stat5-dependent mechanism. A, Increasing concentrations of EGF result in increased type II $\beta$-HSD reporter activity. HeLa cells were cotransfected with a $\text{−301} \rightarrow +45$ fragment of the type II $\beta$-HSD promoter fused to a CAT reporter gene ($\text{−301 CAT}$; 5 $\mu$g), ovine Stat5 (5 $\mu$g), $\beta$-galactosidase (0.5 $\mu$g), and control DNA for a total of 15.5 $\mu$g using the calcium phosphate precipitation method, followed by treatment for 24 h with increasing doses of EGF (0−100 ng/ml). B, hStat5A and hStat5B isoforms in activation of $\beta$-HSD reporter activity. HeLa cells were cotransfected with a $\text{−301} \rightarrow +45$ fragment of the type II $\beta$-HSD promoter fused to a CAT reporter gene ($\text{−301 CAT}$; 5 $\mu$g), hStat5A, or hStat5B (5 $\mu$g), $\beta$-galactosidase (0.5 $\mu$g), and control DNA for a total of 15.5 $\mu$g using the calcium phosphate precipitation method, followed by treatment for 24 h with EGF (25 ng/ml). The number above the bar is fold activation compared with the identically transfected group minus EGF. C, A Stat5 regulatory element confers EGF responsiveness to the human type II $\beta$-HSD promoter region. HeLa cells were cotransfected with $\text{−301}$ (wild-type) or $\text{−301}$ (mutant) CAT reporter constructs (5 $\mu$g), ovine Stat5 (5 $\mu$g), $\beta$-galactosidase (0.5 $\mu$g), and control DNA for a total of 15.5 $\mu$g using the calcium phosphate precipitation method, followed by treatment for 24 h with EGF (25 ng/ml). Data represent the mean ± SE of triplicate cultures after correction for transfection efficiency from a representative experiment of two performed. [Reproduced from F.A. Peltus et al.: Endocrinology 144: 1847−1853, 2003 (125), copyright 2003, The Endocrine Society.]
other nonsteroid factors, which are known to activate other intracellular signaling pathways on steroid-regulated transcription? With these new mechanisms, each of these questions will need to be explored to characterize the essential processes.

5. IL-4. Immune cell populations in the ovary change during the reproductive cycle and cytokines from these immune cells have been shown to affect steroidogenesis (140). Recent data indicate that IL-4 stimulates 3\(\beta\)-HSD mRNA levels in primary cultures of human granulosa-lutein cells (141). IL-4 has been shown to primarily activate Stat6 through Stat6 regulatory elements, one of which is present in the human HSD3B2 promoter at \(\sim 160\) to \(\sim 151\) (Fig. 9). However, the activation of Stat protein signaling often involves multiple Stat proteins with some overlap in function (113). Although IL-4 stimulation is associated with Stat6 activation, IL-4 stimulation of HSD3B2 gene promoter activity requires both an intact Stat5 and an intact Stat6 regulatory element (141).

6. GATA proteins. The GATA proteins are a family of zinc finger transcription factors that bind to GATA regulatory motifs (A/TGATAA/G) in the promoter regions of numerous target genes. Although they were originally identified as crucial regulators of hematopoietic cell differentiation and heart development, the expression of GATA factors is not limited to these two tissues. Interestingly, transcription factors belonging to the GATA family are emerging as novel regulators of steroidogenesis. Indeed, members belonging to this family, namely GATA-4 and GATA-6, are strongly expressed in steroidogenic cells of both the fetal and adult adrenals and gonads (142). In these tissues, several target genes for GATA factors have been identified, such as StAR (143–146), CYP11A (P450 side-chain cleavage) (147), SULT2A1 (161), CYP17 (17\(\beta\)-steroid hydroxylase) (147–150), HSD17B1 (17\(\beta\)-HSD type 1) (151), CYP19 (aromatase) (143), human HSD3B1 (3\(\beta\)-HSD type 1) (152), and most recently human HSD3B2 (3\(\beta\)-HSD type 2) (153). The human HSD3B2 promoter contains four consensus GATA elements within the first 1000 bp upstream of the transcription start site and is potently activated by both GATA-4 and GATA-6 in cooperation with the nuclear receptors SF-1 (NR5A1) and liver receptor homolog-1 (NR5A2). This suggests that GATA factors are key regulators of this gene and that deregulated GATA expression and/or activity might be relevant to pathological processes associated with aberrant HSD3B2 expression such as adrenal insufficiency, male pseudohermaphroditism, and polycystic ovary syndrome (PCOS).

B. Future directions in transcriptional regulation

One area of future studies will be to follow up on new developments in the orphan nuclear receptors SF-1 and DAX-1. The importance and effectiveness of these factors in the regulation of the 3\(\beta\)-HSD expression as well as emerging information on their action can be applied to developing insights into the regulation of 3\(\beta\)-HSD and its potential interaction with other intracellular signaling pathways. For example, there is a growing body of evidence suggesting that SF-1 interacts with other transcription factors such as Egr-1 (111). In addition, SF-1 has been suggested to interact with other transcription factors (154) as well as coactivators and integrating proteins (155, 156). As patterns of interactions
emerge, they may provide insight into mechanisms important for understanding the regulation of 3\(\beta\)-HSD.

Another potential area of future interest relates to regulation by members of the TGF\(\beta\) family. Members of the TGF\(\beta\) family including TGF\(\beta\) have been shown to regulate 3\(\beta\)-HSD mRNA levels in adrenal cells (157). Another potential area of interest relates to regulation by members of the nerve growth factor-induced clone B (NGFI-B) family. Nur77, a member of the NGFI-B family, was shown to regulate type II 3\(\beta\)-HSD mRNA levels in granulosa (158), as well as Leydig and adrenal cells (619). Nur77 stimulates transcription of \(HSD3B2\) through action at a NBRE site (\(-124/-131\) bp from transcription start site) on the promoter, and is only minimally affected by SF-1. Furthermore, Nur77 synergizes with SRC coactivators (SRC-1/2/3) to further enhance \(HSD3B2\) promoter activity in Leydig and adrenal cells (619). Taken together, the identification of Nur77 as a regulator of \(HSD3B2\) expression helps better define the tissue-specific and hormonal regulation of \(HSD3B2\) gene in steroidogenic cells.

C. Regulation of placenta/peripheral tissue type I 3\(\beta\)-HSD

The \(HSD3B1\) gene encoding the type I 3\(\beta\)-HSD shares a relatively high degree of homology with the \(HSD3B2\) gene, not only over the coding sequence but also over the promoter and 5' flanking sequence (Fig. 9). Long stretches of the promoter up to approximately 1 kb upstream of the transcription start site share more than 80\% nucleotide sequence homology with the \(HSD3B2\) promoter. Although this degree of homology might suggest some common regulatory mechanisms, very small changes in critical core nucleotides of regulatory elements can disrupt their function. Therefore, careful functional studies will be needed to map the critical regulatory elements of the \(HSD3B1\) gene and to compare and contrast these elements to those of its homologous \(HSD3B2\) gene.

1. Placenta. Initial studies of the \(HSD3B1\) gene in placental cells indicated that stimulation with phorbol esters increased gene expression and that this effect could not be blocked with protein synthesis inhibitors (159). The regulatory elements important for transcription of the \(HSD3B1\) gene in human placental cells have not been fully mapped. Some studies have suggested that some of the important regulatory elements for this gene are localized in the first intron of the \(HSD3B1\) gene (160) with an element overlapping an Sp1 motif. Recently, a 53-bp placentalspecific enhancer element located between \(−2570\) and \(−2518\) of the \(HSD3B1\) promoter was identified. Within this 53-bp element, two potential placental transcription factor binding sites were found, one identified as being a specific binding site for the transcription enhancer factor-5 (TEF-5), which is highly expressed in human placenta, and the other being an overlapping binding site specific for a GATA-like protein. Because the expression of \(P450sc\) and \(HSD3B1\) is essential for the biosynthesis of PROG, one would assume that their expression is coordinated. It will be of interest in future experiments to investigate whether TEF-5 and GATA proteins also determine the human placental-specific expression of \(P450sc\) (152). Additional studies are needed in this area, particularly using normal, physiological models. In this regard, it is interesting that expression of type I 3\(\beta\)-HSD mRNA levels increases during placental development, during the process of syncytiotium formation (161). If this process involves transcriptional changes, this may provide a model for investigations of the critical regulatory elements in placental expression of 3\(\beta\)-HSD.

2. Crucial role of IL-4 and IL-13 in peripheral tissues. In animal species as well as in humans, there are several reports suggesting that cytokines can regulate the expression of several steroidogenic enzymes. For example, IL-1, IL-2, or IL-3 and TNF-\(\alpha\) exhibit either stimulatory or inhibitory effects on steroid production in ovaries (140, 162–164). IL-1, IL-4, IL-6, IGF-I, and IL-2 modulate \(\beta\)-HSD activity in breast cancer cells lines (165–167). And finally, IL-1 and IL-6 regulate aromatase expression in breast cancer cells and in adipose tissues (168, 169).

IL-4 is produced by T cells, mast cells, and basophils and exerts its activities on many immune cells, including B and T lymphocytes, mast cells, and macrophages. The most specific effects of IL-4 are differentiation of T cells to the Th2 phenotype (secretion of IL-4, IL-5, IL-6, IL-10, and IL-13), and Ig class switching to IgE in B cells (reviewed in Ref. 170). On the other hand, the IL-4 receptor (IL-4R) is also expressed in certain nonimmune cells, thus suggesting that IL-4 may regulate some functions within those cells.

It has recently been shown that IL-4 and IL-13 induce 3\(\beta\)-HSD type I gene expression in ZR-75-1, T47-D, and MDA-MB-231 breast cancer cells; in HT-29 colon and ME-180 cervical cancer cell lines; in the HaCaT immortalized keratinocytes; as well as in normal human mammary and prostate epithelial cells in primary culture (Figs. 12 and 13) (51, 52, 171). The IL-4-induced expression of the \(HSD3B1\) gene requires new mRNA synthesis, but not new protein synthesis, thus suggesting that this induction takes place at the transcriptional level by activating latent transcription factors (Fig. 12) (51).

a. STAT6 activation. Studies of IL signal transduction have clarified the mechanism by which IL-4 functions. Stat6 is an IL-4-activated transcription factor (172, 173). Indeed, recent experiments performed on Stat6-deficient mice demonstrated that Stat6 plays an essential role in IL-4 and IL-13 signaling (174–176). Analysis of the sequences upstream of the \(HSD3B1\) gene revealed the presence of two sequences matching the Stat6 consensus sequence (TTCNNNNGAA) (Fig. 9). The distal site (Stat6#1) is located at position \(−847\) to \(−838\), and the proximal site (Stat6#2) is located between \(−156\) and \(−137\). Stat6 binds in an IL-4-dependent fashion to the 3\(\beta\)-HSD type 1 Stat6#1 and Stat6#2 probes as revealed using EMSA. No such complexes were formed on mutated probes containing two single-base pair substitutions in the Stat6 consensus sequence at positions that disrupt Stat6-DNA binding (51). IL-4 activated Stat6 in all cell lines studied in which IL-4 also induced 3\(\beta\)-HSD type I expression, but not in those cell lines that failed to respond to IL-4 (51, 52). Furthermore, the lack of a stimulatory effect of IL-4 on 3\(\beta\)-HSD type I expression in some cell lines cannot be explained by the absence of Stat6 expression, because this protein was expressed in the IL-4 nonresponsive PC-3, Caco-2, JAR, and JEG-3 cell lines. Rather, our study shows that the cell-specific
action of IL-4 correlates with the activation of Stat6 (Fig. 13) (52).

To determine whether Stat6 DNA-binding activity was modulated by other transduction pathways (see Section V.C.2.b), ZR-75-1 cells were incubated with IL-4 in the presence or absence a phosphatidylinositol 3-kinase (PI3-K) inhibitor (wortmannin), a MAPK inhibitor (PD98058), or an activator of protein kinase C (PKC) pathway [phorbol-12-myristate-13-acetate (PMA)]. It has been demonstrated that neither wortmannin nor PD98059 blocked the induction of Stat6 DNA-binding activity by IL-4, whereas PMA did not change the potency of IL-4 to induce Stat6 DNA-binding activity (177). Those results indicated that Stat6 is specifically activated by IL-4 and independently of the PI3-K, MAPK, and PKC pathways. The fact that: 1) Stat6 plays an essential role in IL-4 signaling, 2) Stat6 DNA-binding sequences are present in the HSD3B1 promoter, and 3) Stat6 is activated by IL-4 in all cell lines studied where IL-4 induced 3β-HSD type 1 expression, but not in those cell lines that failed to respond to IL-4, suggests that Stat6 could be involved in the signal transduction leading to the induction of 3β-HSD type 1 expression by this cytokine. However, we have never been able to show induction by activated-Stat6 of a reporter gene under the control of the HSD3B1 promoter, so it was considered that other pathways could be involved for the effect of IL-4.

b. Multiple signal transduction pathways. There is evidence that IL-4 stimulates bifurcating signaling pathways in which the Stat6-signal pathway is involved in differentiation and gene regulation, whereas insulin receptor substrate (IRS) proteins mediate the mitogenic action of IL-4 (178, 179). The cytoplasmic protein IRS-1 was first identified as a major substrate for the insulin receptor and IGF-I receptor, whereas IRS-2 (also designated 4PS, IL-4 phosphorylated substrate) was first identified as a substrate for the IL-4R (reviewed in Ref. 180). However, both proteins share extensive structural and functional identities and are tyrosine-phosphorylated in response to IL-4, insulin, and IGF-I (181, 182). Moreover, it has been demonstrated that activated IRS-1 and IRS-2 act as mediators of IL-4 mitogenic signaling (178, 179, 182). Despite the inhibitory effects of IL-4 on estradiol (E2)-induced proliferation of ZR-75-1 cells (183), we still decided to investigate whether IRS proteins are involved in the induction of 3β-HSD type 1 expression by IL-4. Our study demonstrated that IL-4 caused rapid tyrosine phosphorylation of both IRS-1 and IRS-2, which was readily detectable after a 15-min exposure (177). The phosphorylation of IRS proteins by IL-4 raised the possibility that this pathway could be involved in IL-4 signaling in ZR-75-1 cells.

To determine their involvement, the effect of other factors that induce the phosphorylation of IRS proteins on 3β-HSD...
type 1 expression was examined. IRS-1 and IRS-2 are also tyrosine phosphorylated by insulin and IGF-I in ZR-75-1, MCF-7, and T47-D breast cancer cell lines (184). As shown in Fig. 8, IGF-I and insulin increase the stimulatory effect of IL-4 on 3β-HSD activity. However, neither IGF-I nor insulin, even at high concentrations, induced 3β-HSD activity in the absence of IL-4, thus indicating that phosphorylation of IRS proteins is not sufficient to induce 3β-HSD type 1 expression.

Because both IGF-I and insulin exerted their stimulatory action on IL-4-induced 3β-HSD activity at EC50 values in the low nanomolar range (177), and knowing that there is no significant binding of insulin to the IGF-I receptor or of IGF-I to the insulin receptor at concentrations less than 10 nM (185), these data suggest that both factors exert their potentiating action through the binding to their specific receptors.

However, as illustrated in Fig. 14, incubation with PMA increased the maximal stimulatory effect of IL-4. It is interesting to note that PMA, even at high concentrations, was unable to induce 3β-HSD activity in the absence of IL-4. Thus, similar to the IRS pathway, activation of the PKC pathway is not sufficient to induce 3β-HSD type 1 expression, but it suggests that at least one signaling molecule involved in signal transduction of the IL-4-induced 3β-HSD type 1 expression is also a substrate for PKC.

Another means of addressing the role of IRS proteins in the induction of 3β-HSD type 1 expression was to study the involvement of pathways downstream of IRS proteins in IL-4 signaling. To study a possible role in the induction of 3β-HSD type 1 expression by IL-4, specific inhibitors of the PI3-K and MAPK pathways were used. Wortmannin and LY294002 are two structurally unrelated molecules that specifically inhibit PI3-K and MAPK pathways, respectively. Wortmannin and LY294002 completely blocked the IL-4-induced 3β-HSD activity. Because it has been shown that the regulatory subunit of PI3-K associates with phosphorylated...
IRS proteins in ZR-75-1 (184), the blockade of IL-4 signaling by the PI3-K inhibitors indicates not only the involvement of PI3-K but also the involvement of IRS proteins in IL-4-induced 3β-HSD type 1 expression.

Phosphorylated IRS-1 and IRS-2 can activate the MAPK pathway by binding Grb2 and engaging the Ras/Raf pathway (186–188). The specific inhibitor of MAPK kinase activation, PD98059, inhibits the induction of 3β-HSD expression by IL-4 (177). Moreover, MAPK activity was also found to be increased in cells treated with IL-4, IGF-I, or insulin compared with untreated cells (177). Although IL-4 failed to activate MAPK in lymphohemopoietic cells (189–191), the activation of MAPK in ZR-75-1 cells is in accordance with reports showing that MAPK is activated by IL-4 in human keratinocytes (192) as well as in breast cancer cell lines (184). Wortmannin is able to block the induction of MAPK by all three factors, thus suggesting that MAPK activation is a downstream effector of PI3-K that is activated by IL-4, insulin, or IGF-I (177). In conclusion, the involvement of IRS proteins in the regulation of HSD3B1 gene expression is supported by the fact that: 1) IL-4 induces the phosphorylation of both IRS-1 and IRS-2; 2) IL-4 activates the MAPK pathway by an PI3-K-dependent manner; and 3) inhibition of downstream targets of IRS proteins (PI3-K and MAPK pathway) block IL-4-induced 3β-HSD type 1 expression.

In accordance with this model, IGF-I and insulin increase the pool of receptors able to phosphorylate IRS proteins and activate MAPK in a PI3-K-dependent manner. Our observation that PMA caused a greater potentiating effect of IL-4-induced 3β-HSD expression than that exerted by IGF-I or insulin may be explained, at least in part, by its capacity to activate a different set of PKC isoenzymes, i.e., the diacylglycerol-sensitive PKC isoenzymes (193), whereas IL-4, IGF-I, and insulin activate atypical (diacylglycerol-insensitive) PKC (194).

The inability of IGF-I, insulin, and PMA to induce 3β-HSD activity in the absence of IL-4 indicates that IRS-1 and IRS-2 phosphorylated proteins and their downstream effectors may cooperate with another IL-4-specific signaling transduction pathway. Thus, it has been postulated that IL-4-induced HSD3B1 gene expression requires the independent activation of at least two transcription factors, i.e., Stat6, an IL-4-specific transcription factor, and a second unidentified factor, which can be activated not only by IL-4 but also by IGF-I, insulin, and PMA. The fact that IL-4-induced Stat6 DNA-binding activity is not affected by wortmannin or PD98059 and that PMA does not modify the potency of IL-4 to activate Stat6 indicates that Stat6 is activated independently of IRS proteins as previously suggested in another model system (195). Taking into consideration these findings, Gingras et al. (196) proposed the following model for the induction of 3β-HSD type 1 induction by IL-4 (Fig. 15).

**D. Species similarity/divergence in mechanisms**

The similarities/differences in the transcriptional control mechanisms between humans and other species will need to be carefully examined. The existence of six different expressed 3β-HSD genes in mice is suggestive of some potentially interesting differences in the mechanisms between the two species because sequence divergence in the promoter regions will likely introduce changes in the control of the isoform genes. Many of these potential similarities/differences will need to be examined using functional studies. Sequences that appear to contain homologous regulatory elements are sometimes nonfunctional in the context of a specific promoter, and sequences that appear...
to have diverged from the core necessary sequences can sometimes prove functional.

The complexity of this process is seen by comparing the potential SF-1 regulatory element in the human HSD3B2 gene promoter with the homologous sequence in the human HSD3B1 gene (placental/peripheral form) and the rat type I gene (gonadal/adrenal form). The human type I gene has a substitution of one nucleotide in the core AAGG sequence resulting in an AAaG core as seen in Fig. 9. A substitution of this kind in the core sequence would predict that the SF-1 element would be nonfunctional in the human HSD3B1 gene. Alternatively, the rat type I gene that is the major gonadal/adrenal form has diverged significantly in sequence from the SF-1 element in the human HSD3B2 gene but maintains the core AAGG sequence. Therefore, it will be important to test the function of these elements because the highly divergent sequence of the rat type I could still be functional.

In a recent study on the expression of the mouse orthologous 3β-HSD, 3β-HSD VI, in giant trophoblast cells during midpregnancy, two transcription factors were identified that determined the trophoblast-specific expression of 3β-HSD VI (197). The two transcription factors, AP-2γ and the homeodomain protein distal-less (Dlx-3), were found in both mouse giant trophoblast cells and human placental JEG-3 cells. In addition, it was reported that AP-2γ was the transcription factor required for mouse trophoblast-specific expression of P450scc. Thus, the identification of an AP-2γ and Dlx-3 binding site in the promoter of the human HSD3B1 gene and the demonstration that these two transcription factor binding sites were located within an enhancer region of the HSD3B1 promoter led to the belief that the placental-specific expression of human 3β-HSD type 1 was determined by the same transcription factors as those found for the trophoblast-specific expression of the mouse 3β-HSD VI (198). However, neither one of these transcription factors is involved in determining the placental-specific expression of human 3β-HSD type 1. As previously mentioned in Section V.C.1, a 53-bp placental-specific enhancer element was identified between −2570 and −2518 of the HSD3B1 promoter. The sequence comprising this enhancer element identified a consensus binding site for a family of TEFs, TEF-5 as well as an overlapping binding site specific for a GATA protein (152). Although the tissue-specific expression of mouse 3β-HSD type VI and human 3β-HSD type 1 are very similar, it may not be surprising that the placental-specific expression is regulated by different transcription factors in the two species. Placental PROG production in humans, which requires 3β-HSD type 1, is absolutely essential for maintenance of pregnancy, whereas in mouse, trophoblast cells have the capacity for PROG production (197) and there is no evidence that the trophoblast-generated PROG is required for maintenance of mouse pregnancy. The difference in the transcription factors that determine placental-specific expression does not appear to be the result of the absence of expression of the

**FIG. 15.** Working hypothesis for the induction of 3β-HSD type I gene expression by IL-4 in ZR-75-1 breast cancer cells. The steps in the pathway known to occur are represented by solid lines, whereas the proposed pathways are shown in dashed lines. A cross on the signaling pathways indicates those that are not involved in the regulation of 3β-HSD type I gene expression. Phosphorylated tyrosines are represented by pY. [Reproduced from S. Gingras et al.: J Steroid Biochem Mol Biol 76:213–225, 2001 (196), copyright 2001, with permission from Elsevier.]
factors identified that mediate human or mouse trophoblast-specific expression. As discussed above, AP-2γ and Dlx-3 are present in human placental JEG-3 cells as well as in mouse trophoblast cells. TEF-5 (199), GATA-2, and GATA-3 (200) are expressed in mouse giant trophoblast cells. Whether GATA-4 or other GATA-like proteins are expressed in mouse trophoblast cells is not currently known (152).

VI. Ontogeny, Localization, and Regulation of 3β-HSD Expression

A. Adrenal

Morphological differentiation during adrenal development results in two functionally distinct organs: the fetal and adult adrenal cortex. In primates, the fetal adrenal cortex consists of an outer neocortical (definitive) zone that is the primary site of aldosterone synthesis, a cortisol-producing transitional zone, and an inner fetal zone that comprises 80% of fetal adrenal volume and is responsible for the synthesis of DHEA and its sulfated derivative DHEA-S. In the adult adrenal, the outer cortex has differentiated through the process of zonation into three steroidogenically and morphologically distinct zones: ZG, ZF, and ZR. In the primate, these zones are responsible for the production of aldosterone, cortisol, and DHEA/DHEA-S, respectively. The ZR is absent in early infancy and starts to develop around ages 5 to 6 before DHEA-S increases steadily until the end of puberty (201, 202). However, in mice and rats, ZG produces aldosterone, ZR and ZF produce corticosterone, but no production of androgens is observed due to the absence of P450c17 expression. In fact, after castration of rats, guinea pigs, and mice, no androgens can be detected in the serum, suggesting that sex steroids are solely of gonadal origin in subprimate species (203).

The onset of 3β-HSD expression in the fetal adrenal cortex correlates with the ability of the definitive zone to synthesize aldosterone and also allows for cortisol production by transitional zone cells due to coexpression of P450c17 (204–207). Although 3β-HSD is not expressed to a high degree in the fetal zone, P450c17 is expressed, thereby directing the steroidogenic pathway toward Δ5-hydroxysteroid (i.e., DHEA/DHEA-S) production. This zone-specific steroid secretion pattern is dependent on the relative expression levels of 3β-HSD, P450c17, and P450 21α-hydroxylase (P450c21) that serve as molecular markers of the adrenocortical developmental state. After birth, the neocortex differentiates into the three functional zones of the adult adrenal cortex. The coexpression of 3β-HSD and P450c21 in the ZG leads to aldosterone production, whereas the coexpression of 3β-HSD and P450c17 in the ZF results in the production of cortisol. The expression of P450c17 in the ZR along with the low levels of 3β-HSD expression leads to the synthesis of DHEA and DHEA-S. A third period of adrenal functional differentiation, in which DHEA/DHEA-S serum levels increase, occurs in humans at around age 6–8 yr. This increase in adrenal androgen production is termed adrenarche and correlates with a decline in 3β-HSD expression in the ZR (Fig. 16). The mechanisms underlying the variability of expression are not well understood at this time, and a deeper understanding of the factors involved in the regulation of 3β-HSD is critical to elucidating the control of adrenal steroidogenesis.

1. Ontogeny and localization. The primate fetal adrenal is derived from the dorsal coelomic epithelium. At 8 wk gestation in the human, the fetal adrenal is composed of chromaffin cells that later form the medulla, fetal, and neocortical zones. The neocortical zone further divides into the ZG, ZF, and ZR, whereas the fetal zone regresses after birth. The fetal zone, which expresses P450c17, catalyzes DHEA synthesis, which serves as a reservoir of precursors for placental estrogen biosynthesis (208). The neocortex also expresses P450c17, as well as 3β-HSD and P450sc, and is the site of fetal cortisol biosynthesis (209). The fetal and neocortical zones have undetectable 3β-HSD protein and mRNA expression at 17–22 wk gestation in humans, whereas P450sc and P450c17 are detected in the fetal and transitional zones (207). At 22 wk gestation, the neocortex starts expressing 3β-HSD, and at 28 wk the enzyme is widely distributed in the neocortex (204). Another study showed that neocortical expression of 3β-HSD could be detected as early as 11 wk gestation, followed by a reduction in expression until 24–25 wk gestation (210). By 35 wk gestation, 3β-HSD expression is observed in differentiated ZG and ZF cells in the mature human fetus and is also seen in the 2- and 8-month-old infant. In the 2-yr-old infant, 3β-HSD staining is seen in all cortical layers (204). Adult adrenal 3β-HSD expression was not seen in the macaque reticularis (211), whereas its expression was low in human reticularis cells accounting for their increased output of DHEA/DHEA-S (212). The highly differential expression of 3β-HSD vs. P450sc and P450c17 in the fetal adrenal is
suggestive of unique transcriptional control mechanisms between these steroidogenic enzyme genes.

In the rhesus monkey, 3β-HSD has been localized to the definitive zone of the fetus between 109 and 148 d, whereas at term (155–172 d), 3β-HSD was localized in both definitive and transitional zones (207). Similarly to humans, rhesus monkey 3β-HSD is contained in the outer regions of the adrenal during fetal life. At midgestation, the levels of 3β-HSD increase, which accounts for a rise in the levels of cortisol in circulation (213). In addition to cortisol output by the fetal adrenal, estrogen levels (via conversion of DHEA) and PREG levels fall after fetalectomy in baboons. This suggests that the fetus is directly responsible for steroidogenesis either by fetal steroid production or via secretion of steroidogenesis promoting factors (214).

Detailed ontological studies in the rat have also been performed. 3β-HSD activity studies in the rat adrenal were initially performed using histochemical techniques (215). These studies revealed that 3β-HSD protein and mRNA were not detected in cortical cells until d 16 of gestation. Differential levels of expression could be seen between d 18 and 21 of gestation with high levels of expression in ZF/ZR relative to ZG. Fetal ACTH secretion begins on d 17–18 in the rat, which could account for this induction in ZF/ZR (216, 217). This pattern was maintained until 15 d after birth. Twenty-five-day-old rats then showed adult expression patterns with equal cytoplasmic 3β-HSD immunostaining and in situ hybridization patterns in all three cortical zones and no staining in the medulla (218). Other studies have shown by immunohistochemistry that 3β-HSD is present in the rat ZG at low levels in the first few days after birth, peaks at d 20, and then decreases to levels below that seen in the ZF until adulthood at 65 d. In the rat ZF, 3β-HSD staining was high at birth and reached a maximum at 14 d, which was maintained until adulthood. The inner part of ZF/ZR was low before d 20 and then increased to ZF levels and remained so until d 90. Aldosterone secretion paralleled the development of the ZG, whereas corticosterone levels were low for the first 14 postnatal days (219, 220). These data show that 3β-HSD expression is expressed in the ZF/ZR before the ZG. Earlier induction of ACTH relative to angiotensin II (A-II) (221) could account in part for this differential expression.

Ontological studies have also been performed in other animals besides rodents and primates. Adrenal studies in sheep demonstrated 3β-HSD localization to the outer cortical layer from d 43 of gestation until term. 3β-HSD localization was observed in ZF from d 65–130 (222). Adult ovine adrenal ZG stained negatively for P450c17 and only faintly positive for 3β-HSD, whereas the adjacent layer of cells (ZF) stained strongly for P450c17 and 3β-HSD (223). Bovine adrenal development studies show that 3β-HSD appears in a narrow ZG/ZF band at d 80 of gestation (224), and that 3β-HSD mRNA expression is seen in the bovine fetal adrenal with higher expression in early and late gestational stages relative to midgestation (225). Species-specific differences must therefore be taken into account when examining 3β-HSD ontology in the adrenal.

2. Regulation

a. ACTH and A-II. The differential expression of the enzymes required for zonal-specific steroid production in the adrenal is under the control of multiple factors. Regulation of 3β-HSD expression in the adult adrenal has long been known to involve ACTH in ZF/ZR and A-II in the ZG. The involvement of ACTH in 3β-HSD induction in the fetal adrenal has been examined. The release of ACTH resulting from metyrapone treatment (an inhibitor of 11β-hydroxylase activity) of rhesus monkeys at 135–137 d gestation caused an induction of 3β-HSD in the transitional zone suggesting that ACTH plays a role in the induction of 3β-HSD at term (226). The transitional zone, which is comprised of cortical cells that express P450c17 and 3β-HSD (i.e., cortisol production), and the definitive zone, which lacks P450c17 expression but does express 3β-HSD (i.e., mineralocorticoid production), develop in the late stages of gestation. It has been shown by immunocytochemistry that induction of 3β-HSD and P450c17 in the transitional zone of baboon adrenals occurs late in gestation and is dependent on ACTH. However, the development of the definitive zone at midgestation occurs in the absence of ACTH (227). ACTH receptor and 3β-HSD mRNA and protein levels were also decreased in the developing definitive zone of the fetal adrenal. These data suggest that ACTH is required for the up-regulation of ACTH responsiveness and steroidogenesis in the fetal definitive zone of the baboon adrenal gland (228). Additional evidence for adrenal differentiation in the absence of ACTH comes from studies of anencephalic human fetuses that lack ACTH production, yet in which the definitive zone appears to develop normally (229). In the fetal sheep adrenal, ACTH administration in the late stages of pregnancy led to an increase in P450c17 but not 3β-HSD, suggesting that control of 3β-HSD levels in the pregnant sheep is more complex (230). These results suggest that the ontogenesis of 3β-HSD expression in the transitional zone is dependent on ACTH, yet these effects appear to be species specific.

The functional development of the rat adrenal cortex is characterized by a triphasic response to ACTH with a nadir in responsiveness around neonatal d 10. Immunoactive levels of 3β-HSD are low until d 10 relative to adults, although enzymatic activity is similar to adults. ACTH injection on d 10 increased 3β-HSD staining (231). Studies in adult rats have shown that 3β-HSD staining by in situ hybridization followed a uniform pattern throughout the adrenal cortex that was unaffected by ACTH injection until responsiveness appeared at d 4 of treatment (232). Other studies have been performed using chronic stimulation (injections twice daily for 9 d) of adult rats with ACTH. Results from these studies showed increased StAR mRNA levels, but decreased 3β-HSD and angiotensin type I receptor transcripts in ZG cells, whereas the levels of P450scc and P450C21 mRNA did not differ significantly from the control values, thus suggesting a control of StAR expression by ACTH and 3β-HSD by A-II. Interestingly, Western blotting analysis revealed that StAR protein levels were increased, whereas 3β-HSD protein levels were unchanged. In addition, ACTH suppression via dexamethasone (DEX) treatment for 5 d did not affect either StAR or 3β-HSD. In the ZF/ZR, chronic ACTH stimulation led to increases in StAR, whereas 3β-HSD was unchanged (233). These data demonstrate that: 1) the ability of ACTH to stimulate 3β-HSD follows a temporal pattern in the rat fetal adrenal; 2) differential regulation of steroidogenic enzymes...
exists under chronic exposure of adult adrenals to circulating ACTH; and 3) other factors are involved in modulating 3β-HSD expression.

Primary cultures of human adrenal cells have demonstrated the capacity of ACTH and A-II to regulate 3β-HSD. In vitro studies of fetal zona cells demonstrate that they are responsive to ACTH with an induction of 3β-HSD, suggesting that an in vivo endogenous factor is inhibiting 3β-HSD production (205, 206, 234). Many studies have shown increased receptivity of adrenal cells after the addition of ACTH and A-II as well as second messenger mimetics of ACTH (cAMP/forskolin/cholera toxin induce PKA activity) and A-II (phorbol esters induce PKC activity). In human reticularis-fasciculata cells, the stimulatory effects of ACTH and A-II were additive only on 3β-HSD expression (235). Moreover, in rat adrenal cells in the absence of ACTH, this enzymatic activity was found to decay with a half-life of 3.1 d, which was similar to the half-life of the enzyme activity induced by ACTH in vitro (3.5 d) (236). The induction of 3β-HSD activity by ACTH or dibutyryl cAMP required a lag period of approximately 4 h and was dependent on RNA and protein synthesis (236). ACTH and cAMP also up-regulate 3β-HSD protein and mRNA expression in cultured bovine adrenocortical (BAC) cells (157, 237), yet A-II treatment decreases ACTH-induced 3β-HSD expression in BAC cells (157, 238). These in vitro model systems have been useful in the examination of ACTH and A-II on steroidogenesis regulation.

Two steroid-producing adrenal cell line models exist: H295R (human adrenocortical carcinoma) and Y1 (mouse adrenocortical carcinoma). In H295R cells, it has been demonstrated that forskolin, A-II, and cAMP treatments stimulated 3β-HSD mRNA; however, this effect could be blocked by cycloheximide treatment (239, 240). H295R studies have also shown that cAMP and phorbol esters induce 3β-HSD (240). K+, an agent known to increase intracellular Ca2+ through the opening of voltage-sensitive Ca2+ channels, caused an increase in H295R cells of P450c17 and P450scc, but not of 3β-HSD mRNA levels. This suggests that phorbol ester effects in this system may signal via a pathway independent of that induced by Ca2+ alone (241). Interestingly, staurosporine (PKC inhibitor) increases 3β-HSD mRNA levels in mouse Y1 cells, suggesting that PKC is a tonic inhibitor of steroidogenesis in these cells (242). The effects of induction of PKC activity via the employment of phorbol esters may not be indicative of signaling by the angiotensin type 1 receptor due to the differential effects seen in these model systems.

b. TGFβ, IGF, and growth factors. Because the expression of 3β-HSD during adrenal development is not completely under the control of ACTH and A-II, other factors must be involved in the induction and repression of 3β-HSD in the neocortical zone and the fetal zone, respectively. Candidate molecules involved in this regulation include EGF, fibroblast growth factor (FGF), IGF, and TGFβ. EGF and FGF are mitogenic in human fetal adrenals (243), whereas aFGF and bFGF have been shown to be mitogens in BAC and Y1 cells (244, 245). In addition, it has been postulated that the mitogenic effects of GH in the adrenal could be due to IGFs (246). On the other hand, TGFβ has a growth inhibitory effect (247). Therefore, there appears to be a complex interplay of factors controlling adrenal growth and development, and combinations of these factors could be involved in the regulation of 3β-HSD and other steroidogenic enzymes in vivo.

Several studies have implicated EGF in the regulation of adrenal development and function. Macaque fetuses treated with EGF show induced expression of 3β-HSD protein in the transitional zone of the fetal adrenal, whereas betamethasone treatment resulted in a reduction of 3β-HSD protein levels (248). Studies by McAllister and Hornsby (249) have shown that forskolin induction of 3β-HSD activity in human adrenocortical cells could be enhanced by A-II, EGF, or FGF treatment, whereas other work has shown that EGF can induce 3β-HSD mRNA and cortisol production in H295R cells (125). Another study has shown that infusion of EGF into ewes for 24 h resulted in a 700% increase in cortisol levels (250). Activation of the EGF receptor is probably not being mediated by EGF in humans because EGF is not expressed in the adrenal cortex. However, TGFα is expressed (251). These data suggest that activation of the EGF receptor in the adrenal cortex can induce steroidogenic enzymes including 3β-HSD, but the precise role of EGF and EGF-like ligands is unclear.

Another factor thought to play a role during adrenal development is TGFβ. Antisense oligonucleotides to TGFβ caused a 2-fold increase of mRNAs for 3β-HSD and P450c17 in BAC cells (252). Similarly, TGFβ decreased P450c17 and 3β-HSD expression, but the effect was more pronounced on P450c17 levels in the cow (157). Interestingly, human adrenocortical cells treated with TGFβ1, in the presence of ACTH resulted in an increase in 3β-HSD mRNA levels, a decrease in P450c17 mRNA, but no effect on P450scc expression (253). Studies by Naville et al. (254) showed that ACTH up-regulates 3β-HSD mRNA and protein expression in ovine adrenocortical cells, whereas P450c17 and 3β-HSD expression were decreased by TGFβ. However, the effect TGFβ was more pronounced on P450c17 levels in the sheep. Furthermore, TGFβ1 decreased 3β-HSD mRNA and protein levels in mouse Y1 cells (255). Therefore, TGFβ may play an important role in the down-regulation of 3β-HSD and steroidogenesis in the adrenal gland, however the regulatory context in which TGFβ exerts its effects may be lost in vitro.

Insulin and IGFs have been known to enhance steroidogenesis in multiple tissues, and these effects have been shown to involve the up-regulation of 3β-HSD mRNA levels. IGF-II enhances ACTH-stimulated, but not basal levels of 3β-HSD mRNA in cultured human fetal adrenals (256). Treatment with insulin and IGF-I increased 3β-HSD and P450c17 mRNA levels in human adrenocortical cells, and this increase was coincident with a decrease in the DHEA/cortisol ratio (257). Although P450c17 and 3β-HSD mRNAs were increased by IGF-I/II treatment, P450scc levels remained unaffected in human adult fasciculata-reticularis cells. In addition, the acute steroidogenic response to ACTH of cells pretreated with IGF-I, IGF-II, or insulin was 3- to 6-fold higher than that of control cells (258). ACTH and IGF-I also increased 3β-HSD and P450c17 mRNA levels in BAC cells (258). Therefore, these studies demonstrate that insulin and IGFs play a role in enhancing adrenal steroid output, and that part of this effect is mediated at the level of 3β-HSD.
c. Steroids. Data from multiple studies have suggested that steroids can regulate adrenal 3β-HSD and possibly steroidogenesis. If verified, this would have important implications on the ability of steroids downstream of 3β-HSD catalysis to feedback or feed-forward on steroid production. For example, DEX can enhance 3β-HSD mRNA levels and cortisol production in H295R cells, and this effect is enhanced with PMA treatment (125). These results suggest that cortisol production by the ZF might enhance the steroidogenic potential of the adrenal gland through direct transcriptional effects. Other work has shown that E2 decreased 3β-HSD activity in a dose-dependent manner in H295R cells (259). The PROG/glucocorticoid receptor antagonist RU486 administered to hypophysectomized (HYPOX)/castrated/ACTH-replaced rats decreased 3β-HSD activity, yet corticosterone levels were unaffected (260). 3β-HSD activity is greater in female than male adrenals of C57BL/6j and C3 h/Hej mice. Injection of T or DHT decreased adrenal 3β-HSD activity in both strains (133). These studies therefore open the possibility for complex feedback by androgens, estrogens, and glucocorticoids in the regulation of adrenal steroidogenesis.

d. Other regulatory mechanisms. Several studies have suggested that adrenal 3β-HSD is regulated by factors other than those mentioned. For example, thyroid hormone (T3) regulation of 3β-HSD has been examined in the adrenal gland as well as in other tissues. T3 alone had no effect, however cotreatment with T3 enhanced ACTH up-regulation of 3β-HSD activity in human fetal adrenocortical cells (261). On the other hand, T3 and T4 reduced 3β-HSD activity in male rat ZFR cells (262). A detailed examination of T3 performed in the perch showed that T3 caused a linear increase of 3β-HSD activity in cells isolated from the ovarian follicles. These studies also demonstrated that T3 induction required protein synthesis, which could be overcome by the addition of T3-induced protein (263, 264). Furthermore, piscine gonadotropin and T3 stimulated 3β-HSD activity in perch testis; however, the kinetics of stimulation differed (265). These studies therefore suggest that the thyroid hormone can also participate in the regulation of steroidogenesis by modulating 3β-HSD activity.

Other factors tested include CRH, prostaglandins, and hypoxemia. CRH increased DHEA-S/cortisol production by cultured human adrenal fetal zone and neocortical zone cells in a dose-dependent manner (266). This suggests a direct role for the pituitary factor in the regulation of adrenal steroidogenesis. Chronic treatment of BAC cells with PGE2 induced 3β-HSD mRNA and protein expression (267). Prostaglandin signaling in the adrenal might therefore be involved in the regulation of 3β-HSD. Finally, hypoxemia increased the cortisol to Δ4-DIONE ratio in pregnant sheep fetuses and also increased adrenal P450sc, P450c21, and 3β-HSD, although P450c17 remained unaffected (268). These factors may therefore play a role in the regulation of adrenal steroidogenesis at the level of 3β-HSD; however, more work needs to be done to determine whether these effects are a general phenomenon or a specific effect of the model systems tested.

3. Adrenarche. Adrenarche is characterized by an increase in adrenal androgen secretion (DHEA/DHEA-S), which is not accompanied by an increase in cortisol and is independent of the gonads or gonadotropins. Adrenarche is limited to Old World primates (269) and occurs at around age 6–8 yr in humans. After this period, P450c17 expression continues in the ZR, whereas that of 3β-HSD declines. Several studies have shown that 3β-HSD expression decreases in the ZR in humans over 8 yr old (Fig. 16) (270–272). However, the mechanisms underlying the decrease in 3β-HSD expression are still unknown. Another proposed mechanism that could explain the increase in DHEA/DHEA-S output during adrenarche is an alteration of the ratio of 17α-OH/17,20-lyase activities of P450c17 (211, 273, 274). Increases in the coupling of the allosteric factor, cytochrome b5, to P450c17 promote 17,20-lyase activity and androgen synthesis by P450c17 independent of increases in P450c17 expression. Moreover, an increase in SULT2A1 expression is observed, which results in an increased conversion of DHEA to DHEA-S, further attenuating Δ4-steroid production (620). Adrenarche may therefore result from an increase in 17,20-lyase activity of P450c17 due to an increase in cytochrome b5 association, an increase in SULT2A1 expression along with a decrease in 3β-HSD expression, which results in an increase of adrenal androgen output.

B. Ovary

At birth, the ovary consists of steroidogenically inert primordial follicles that during adulthood are recruited to proliferate and differentiate into distinct layers with specialized functions. These follicles pass through preantral, antral, and ovulatory stages under gonadotropin and intracrine control in which the outer androgen-producing theca layer and the inner estrogen-producing granulosa layer develop. After ovulation, follicles differentiate into the PROG-secreting CL. Each of these structures possesses a different steroidogenic profile that is the result of altered steroidogenic enzyme expression. Differential regulation of 3β-HSD plays an important role in the steroidogenic profile of ovarian tissue, and a complex interplay of pituitary factors and ovarian factors maintains a tight control over steroidogenic enzyme control. Ontological studies have shown that 3β-HSD is expressed during some fetal stages in human ovaries, but then disappears until puberty. Therefore, fetal human ovaries are steroidogenically quiescent except for a window late in gestation (275), so most estrogens seen by the primate fetus are of placental origin (276). This is in contrast to testicular expression because androgen production by the male embryo is critical for male sexual development (277). In humans, hilar cells of the ovary express 3β-HSD at 26 wk gestation as detected by immunohistochemistry (210), whereas 3β-HSD protein could be seen in the theca and interstitial layers of the human fetal ovary from 28–34 wk. No 3β-HSD staining was seen from birth until puberty (275). The role of steroid production in the human fetal ovary is unclear at this time. In contrast to humans, 3β-HSD protein cannot be detected in the fetal rat ovary and is not seen until 6 d after birth in the developing theca (278, 279). Similarly, 3β-HSD is not expressed in the fetal mouse ovary (280). Steroidogenesis by the ovary does not appear to be critical for female development,
with the caveat that there is an unknown function of steroid production during the development of the human ovary.

1. Follicle

a. Follicular localization. 3β-HSD localization studies track the molecular machinery required for PROG and androgen production by the thecal layer during primate folliculogenesis. Androgens are then further metabolized into estrogens by the granulosa cells (281). 3β-HSD mRNA has been localized to the theca interna of preantral, antral, and atretic follicles as well as the CL (282–285). Preantral/antral expression studies show 3β-HSD mRNA and protein expression in the human ovary to be initially in the theca and then developing in the granulosa layer as folliculogenesis continues. Interestingly, some layers of theca interna cells next to the basement membrane showed no 3β-HSD expression (282, 283). Low levels of 3β-HSD relative to P450c17 in the theca during the proliferative phase of the menstrual cycle lead to more Δ⁵-steroid precursors being produced, which results in higher estrogen output by the follicle (286).

In nonprimate species, 3β-HSD has been shown to have different expression patterns. In the rat, 3β-HSD mRNA is elevated throughout the estrous cycle. Preantral, antral, and preovulatory rat follicles showed 3β-HSD expression in the theca, but no expression was seen in the granulosa layer (287). During gestation, 3β-HSD could not be detected at d 10, but increased on d 14, 17, and 21 (288). Similarly to the rat, 3β-HSD is localized to the theca-interna layer of the pig follicle (289–291). In contrast to rodents, pigs, and primates, 3β-HSD expression in the cow was seen in all stages of the preovulatory follicle in both theca and granulosa layers (292), and mRNA and protein levels peaked in bovine ovaries on d 16–17 after estrous (293). After formation of theca interna around the granulosa cells, mRNA for 3β-HSD is expressed in theca cells about the time of antrum formation, and expression increases with the growth of early antral follicles (294–296). Moreover, granulosa cells of preantral and early antral follicles (less than 4 mm) do not express 3β-HSD, indicating that bovine follicles less than 4 mm in diameter are not able to convert cholesterol to PREG and subsequently to PROG (297). Selection of dominant follicle occurs between 36 and 48 h after initiation of the first follicular wave and is associated with mRNA expression for 3β-HSD in granulosa cells (295, 296, 298). Expression in thecal and granulosa cells decreases dramatically in preovulatory follicles after the preovulatory LH surge (292, 299). Other studies in the cow have localized 3β-HSD mRNA to theca at 12 h after the first follicular wave, and expression in the theca was maximal between 24 and 96 h (298). These studies suggest that the primary site of 3β-HSD expression is in the thecal layer of the follicle, yet expression in the granulosa layer is seen depending upon the stage of folliculogenesis and species examined.

b. Pituitary control. Pituitary hormones are a primary means of regulating steroidogenesis in the ovary. Follicular binding of the gonadotropins FSH and LH causes an increase in cAMP and phosphatidylinositol turnover, which in turn results in an increase in 3β-HSD expression concomitantly with other steroidogenic enzymes under the similar control by PKA and PKC. In vitro studies have shown that human theca-interna cells responded to cAMP with an induction of 3β-HSD (300). Similarly, 3β-HSD activity was increased by cAMP in fetal rat ovaries (301), and 3β-HSD mRNA levels in pseudopregnant rats rose dramatically 2 d after induction of ovulation (302). The induction of rat 3β-HSD by FSH can be attenuated by AG18 (a tyrosine kinase inhibitor), suggesting that an undetermined tyrosine kinase pathway is involved in the 3β-HSD-dependent signal transduction pathway of FSH action (303). Activation of the PKB/Akt kinase pathway by FSH has been reported to occur in terminally differentiated granulosa cells, in a cAMP-dependent and PKA-independent manner (304). In these cells, FSH also activates the p38 (305) and ERK MAPKs (306, 307), indicating that the FSH-receptor owns the structural requirements for coupling to these pathways.

Work in the pig parallels 3β-HSD regulation seen in rats and primates. The porcine granulosa tumor cell line, JC-410, responded to cholera toxin with an increase in 3β-HSD and P450sc mRNA, but not P450sre (308). LH/hCG treatment increases 3β-HSD expression in porcine granulosa cells, which can be attenuated with PMA treatment (309). 3β-HSD mRNA expression increases almost linearly between d 3 and 7 in preovulatory follicles of the pig (290). These studies show that cAMP production induced by gonadotropins is an important regulatory mechanism for the control of 3β-HSD, yet modulation by other factors is evident. Another pituitary factor critical to the regulation of ovarian steroidogenesis in rodents and possibly primates is PRL. Although the direct control of 3β-HSD by PRL is unclear, studies have been performed that suggest its ability to alter 3β-HSD enzyme levels. Martel et al. (310) have demonstrated that induction of 3β-HSD mRNA levels in the follicle of hypophysectomized rats can be accomplished by hCG but not FSH treatment. In addition, PRL was shown to be inhibitory, yet the inhibition could be counteracted with hCG treatment. Additional studies using in situ hybridization showed that the administration of hCG for 9 d induced increases of 146% in 3β-HSD mRNA levels in ovarian interstitial cells of pseudopregnant rats. Treatment with PRL caused a 78% decrease in 3β-HSD mRNA levels in CL after 9 d of treatment (311). Although the levels of PRL used in these studies were high, PRL was effective in the control of ovarian steroidogenesis.

Although declines in serum gonadotropin levels during menopause limit the capacity of the ovary to produce steroids, some steroid production continues. In postmenopausal women, for example, 30% of circulating Δ⁵-DIONE is of ovarian origin (312, 313). 3β-HSD expression continues in menopausal and postmenopausal women and is localized to dispersed interstitial cells (275). These studies suggest that ovarian steroid production in postmenopausal women continues, but the decline in pituitary control dramatically changes the steroid profile. Due to the continued expression of steroidogenic enzymes, the postmenopausal ovary could be viewed as a site of both local synthesis and peripheral conversion of steroids produced by the adrenal.

c. Nonpituitary control. Other factors of extrapituitary origin have been examined for their capacity to regulate follicular 3β-HSD. These factors, which include PROG, IGFs, thecal differentiating factor (TDF), GnRH, EGF, and β-
adrenergic agents, may modulate gonadotropic and lactogenic control of 3β-HSD expression. Furthermore, 3β-HSD autoantibodies are present in patients with premature ovarian failure, potentially affecting ovarian steroid biosynthesis (314). However, the relative role of these factors in controlling follicular steroid production independent of trophic factors is a matter of debate.

PROG is a critical component in the ovulatory process in rodents and primates (315). However, the role that PROG plays in the regulation of steroidogenic enzymes in the ovary is less well defined. Treatment of macaques with trilostane (a competitive inhibitor of 3β-HSD) allows for the examination of 3β-HSD regulation in the absence of PROG and other active steroids. For example, an hCG bolus administered to macaques resulted in a time-dependent decrease in P450scc mRNA, whereas 3β-HSD increased in a steroid-independent manner (316). Although these studies do not implicate PROG as a major inducer of 3β-HSD in primates, the effects on basal production of steroids have yet to be examined.

In the rat, however, PROG does appear to have a positive effect on the transcription of 3β-HSD. Onapristone, which is an antiprogesterin, blocks ovulation and decreases PROG receptor (PR) and 3β-HSD protein expression in pseudopregnant rats (317). Furthermore, RU486 (a PROG/glucocorticoid antagonist) administered before hCG injection in the pseudopregnant rat model attenuated the hCG induction of 3β-HSD activity (318). R5020 (a PROG agonist) and PROG in the presence of the 3β-HSD inhibitor, cyanothene, augmented the FSH induction of 3β-HSD activity in a dose-dependent manner in rat granulosa cells (319). Progesterone may therefore play a role in the feedback regulation of 3β-HSD in the rat ovary.

There is evidence that the IGFs and their binding proteins play a role in ovarian steroidogenesis. Studies in the rat have shown that intrafollicular IGF-I amplifies granulosa cell steroidogenesis in this species (320). Similar to results obtained in the adrenal gland, IGF-I appears to augment trophic hormone stimulation of ovarian steroidogenesis. Cultured rat granulosa cells showed a 3.8-fold basal and 1.8-fold FSH-stimulated increase in 3β-HSD activity and mRNA levels by IGF-I treatment (321). IGF-I also increased 3β-HSD mRNA by 2-fold in theca-interstitial cells isolated from hypoximmature rats (322). The relative importance of an autocrine effect of IGF on ovarian function has yet to be fully determined.

TDF, which is secreted by the preantral follicle, stimulates theca-interna androgen production in a LH-independent manner. TDF is induced by FSH in the rat and increases 3β-HSD, P450scc, P450c17, and LH receptor mRNA levels (323). 3β-HSD mRNA is also expressed in the theca-interstitial layer of the rat follicle before LH-responsiveness. Studies showed that the treatment of dispersed cells from 4-d-old rat ovaries with media from preantral follicles containing TDF activity caused an induction of 3β-HSD and other steroidogenic enzymes in these cells along with an increased androgen production (324). Therefore, FSH-induced TDF may enhance the steroidogenic capacity of the rat follicle.

Other nonpituitary factors shown to have effects on 3β-HSD are GnRH, EGF, and β-adrenergic agonists. The GnRH receptor has been shown to be expressed in the follicle (325). Moreover, the GnRH agonist, buserelin, increased 3β-HSD activity in pseudopregnant rat ovarian cells (326), suggesting a role for GnRH outside of pituitary in the control of ovarian steroidogenesis. Another factor that might have an intravarian role in the regulation of 3β-HSD is EGF. In rat granulosa cells, EGF increases 3β-HSD in the presence or absence of TGFβ and increases FSH induction of this parameter (327). Finally, studies have suggested a role for direct innervation and local production of neurotransmitters within the ovary in the control of 3β-HSD and ovarian steroidogenesis (328, 329). Indeed, terbutaline and isoproterenol (β-adrenergic agonists) produced dose-dependent increases in 3β-HSD mRNA in porcine granulosa cells (330). The regulation of 3β-HSD and ovarian steroid output involves an interplay of multiple factors (intrinsically and extrinsically) of which the complexity has yet to be fully ascertained.

2. CL

a. CL localization. After ovulation, luteinization ensues in which the follicle becomes highly vascularized and secretes copious amounts of PROG. PROG output is controlled in part by the amount of 3β-HSD enzyme. Macaque localization studies demonstrate high levels of 3β-HSD expression in the early luteal cycle with a decline in expression thereafter (331, 332). A more detailed temporal examination showed expression of 3β-HSD mRNA from d 1–9 of an induced cycle in CL of macaques (333). In the sheep, however, 3β-HSD mRNA was not detected in ovine CL until 48 h after onset of estrous (334). These results suggest that 3β-HSD localization to the CL is a marker of the PROG production capacity of the CL.

Several studies have correlated the luteal expression of 3β-HSD with that of androgen receptors (AR) and PR. These receptors may alter luteal function based upon local PROG and androgen concentrations. For example, 3β-HSD, AR, and PR are all expressed in human CL (284, 335). In addition, 3β-HSD and PR, but not ER, were present at the highest levels during the midluteal phase of the baboon menstrual cycle (336, 337). As in the baboon, 3β-HSD and PR show the highest expression levels in the midluteal phase of the macaque cycle (338), and 3β-HSD was also detected in the CL of macaques with coexpression of AR (339). These studies suggest a potential role of steroids in the modulation of 3β-HSD levels, and this potential will be discussed further.

Luteal cells are derived from the theca and granulosa layers of the follicle. Two types of luteal cells have been identified in multiple species: large and small. Large luteal cells contain more 3β-HSD mRNA per microgram than small ovine luteal cells (340). Small, medium, and large 3β-HSD positive cells are also found in rhesus monkeys (341). In the mouse, 3β-HSD staining was seen in large and small luteal cells. Although the functional significance of luteal cell types is unclear, the two cell types are regulated differentially as is the case in the ovine CL (334).

b. CL regulation. The CL is a transient organ that is under the control of luteotrophic factors that maintain PROG output. In primates, LH/hCG action through the LH receptor provides a primary means of luteotropic support. For example, hCG has been shown to maintain 3β-HSD mRNA expression in humans (342) and macaque CL (343). Furthermore, LH, hCG, and forskolin increased 3β-HSD activity in human
granulosa-lutein cell culture (344), and induction of 3β-HSD mRNA was observed by cAMP or hCG treatment of porcine granulosa-lutein cells (309). In addition, FSH has been shown to increase 3β-HSD protein and mRNA levels in human granulosa-lutein cells, and this effect could be enhanced by cotreatment with insulin (345). In rodents, both PRL and LH are important luteotropic factors. PRL potentiated hCG induction of 3β-HSD mRNA in rat CL (346), and treatment with mPL-1 (a placental lactogen) resulted in a dose-dependent increase in PROG production (347). Owing to the luteotropic nature of PRL in the rat CL, it is possible that PRL might play a contributing role in the primate CL. Prolactin has been shown to increase basal PROG production in antral follicles (348), dispersed CL cells (349), and in granulosa-lutein cell cultures obtained from women undergoing egg retrieval in in vitro fertilization procedures (350, 351). Increases in PROG production occurred at physiological doses of PRL, but these effects were reversed when doses approached levels seen in hyperprolactinemic patients (348). Therefore, it is postulated that PRL might be capable of regulating genes involved in the hyperprolactinemic patients (348). Therefore, it is postulated that PRL might be capable of regulating genes involved in the PROG biosynthetic pathway. Although the direct control of 3β-HSD by PRL in humans has yet to be demonstrated, PRL has been shown to up-regulate 3β-HSD transcriptional activity in vitro (122). The activation of LH and PRL receptors is therefore an important event in the maintenance of 3β-HSD activity and PROG secretion by the CL.

As is seen in other steroidogenic tissues, increases in PKA and PKC activities modify 3β-HSD levels in the CL. Interestingly, PKA activity induced by increases in cAMP is affected by cross-talk from PKC pathways mimicked by treatment with phorbol esters. It has been shown that tetradecanoyl phorbol acetate decreased 3β-HSD activity in human granulosa-lutein cells (352). Forskolin treatment increased PROG production in small ovine luteal cells, whereas PMA inhibited PROG production in large and small cells, yet neither PMA nor forskolin altered 3β-HSD activity (353). Other studies have shown that PMA inhibited hCG/cAMP/LH/forskolin induction of 3β-HSD mRNA in porcine granulosa-lutein cells (309). These studies thus suggest that cross-talk exists between PKA and PKC pathways in the control of 3β-HSD expression in the CL.

Although LH is a critical control factor for PROG output, there is evidence that LH may be, at least partially, uncoupled to 3β-HSD mRNA levels. Administration of a GnRH antagonist to macaques suggested an uncoupling of PROG production from LH stimulation. Although PROG secretion was markedly diminished after 24 h of antagonist treatment, there were no differences in P450sc and 3β-HSD mRNA levels between antagonist-treated and control animals. These data suggest that P450sc and 3β-HSD mRNA regulation are uncoupled from LH-induced PROG production (354).

Rothchild (355) and others have suggested that PROG output by the CL could be regulated by endogenous and circulating steroids. Presumably, these intrinsic factors could work independently of external luteotropins at the level of 3β-HSD regulation. For example, triolstane treatment of female macaques reduced PROG production, but not relaxin output by the CL, suggesting that PROG is important in regulating PROG biosynthesis in the primate and this effect is uncoupled from luteolysis (356–358). RU486 induced luteolysis in rats with a decrease in 3β-HSD activity and an increase in 20α-HSD activity (359–362). On the other hand, R5020 but not hydrocortisone increased PROG output of rat luteal cells from d 19 of pregnancy (363). E2 decreased 3β-HSD activity in isolated human (364, 365) and rat (366) luteal cells. Results from these studies suggest interplay in the steroid modulation of PROG output by the CL.

Other factors besides LH/hCG, PRL, and steroids have been shown to affect 3β-HSD regulation in the CL. These include growth factors, LHRH, catecholsterogens, and adrenergic innervation. In the case of growth factors, cAMP-stimulated 3β-HSD activity was enhanced by FGF, EGF, and TGFβ in cultured human granulosa-lutein cells (352), suggesting that these factors may play an autocrine role in the regulation of 3β-HSD in the CL. Similarly, the administration of GH to normally cycling women resulted in an increase of 3β-HSD mRNA levels in granulosa-lutein cells (367), although these effects may be mediated indirectly through the action of IGFs. On the other hand, GnRH antagonist decreased 3β-HSD content in rat luteal cells in culture (368), demonstrating the ability of GnRH to have a direct role in the regulation of luteal PROG production. Finally, catechol- estrogen treatment has been shown to down-regulate 3β-HSD expression in rat luteal cells (369), whereas adrenergic innervation of the cow increases 3β-HSD activity and PROG secretion (370). These studies again suggest that multiple luteotropic agents are involved in the regulation of 3β-HSD activity and PROG output by the CL, yet their relative importance has not been determined.

c. Luteolysis. Luteolytic factors are involved in the controlled destruction of the CL, a process termed luteolysis. Evidence for the induction of luteolysis by prostaglandins is extensive, and evidence also exists for direct regulation of 3β-HSD by PGF2α during this process. A 50% reduction in 3β-HSD mRNA and protein levels was observed 4 h after injection of PGF2α in pseudopregnant rats. This effect was transient because 3β-HSD levels returned to normal 8 h after injection (371). Along with a decrease in 3β-HSD activity, PGF2α injection into whole rats resulted in an increase in 20α-HSD activity, and this effect could be overcome with a dose of PROG on d 19 of pregnancy (363). In isolated porcine granulosa cells, PGF2α caused a reduction of P450sc and 3β-HSD mRNA levels (372). PGF2α has also been shown to induce luteolysis in cows and decrease 3β-HSD expression (373–375). In ewes, PGF2α injection at d 11 of estrus resulted in a 95% reduction in 3β-HSD mRNA after 48 h of treatment in large luteal cells (376). Although the role of prostaglandins in primate luteolysis is unclear, PGF2α is clearly a mediator of luteal regression in other species.

Another factor known to induce luteolysis in rodent and play a role in 3β-HSD regulation is LH. LH-induced luteolysis on d 19 of pregnancy resulted in a decrease in 3β-HSD activity and an increase in 20α-HSD activity (359–362). On the other hand, R5020 but not hydrocortisone increased PROG output of rat luteal cells from d 19 of pregnancy (363). E2 decreased 3β-HSD activity in isolated human (364, 365) and rat (366) luteal cells. Results from these studies suggest interplay in the steroid modulation of PROG output by the CL.
onstrate that LH signaling plays a role in luteal regression in rodents.

3. PCOS. PCOS is an ovarian disorder associated with hyperthecosis of the ovary and elevated serum LH, insulin, and androgen levels. Several studies have put forth evidence of aberrant 3β-HSD regulation in polycystic thecal cells. Troglitazone (an insulin-sensitizing agent) lowers androgen concentrations in women with PCOS. It was also shown that troglitazone reduced PROG production in porcine granulosa cells. In addition, 3β-HSD enzymatic activity was reduced 60% after treatment (379). A reduction in 3β-HSD levels may be involved in the etiology of the condition.

C. Testis

1. Ontogeny and localization. In the human fetus, Leydig cells of the testis secrete T, which promotes differentiation of the Wolffian ducts, urogenital sinus, and external genitalia. Leydig cells first appear at 8 wk gestation in humans. T secretion in humans begins around 8 wk gestation, peaks around 14–16 wk, and then begins to fall, and these low levels are then maintained until late gestation when they decrease even further (383–385). Plasma T levels are high at birth in boys, fall within 2 to 5 d, and then rise again at 2–3 wk of life. They decrease correlatively with time from the third month to the sixth month of life, where minimal levels are reached and maintained until the onset of puberty (386–389). The induction of several steroidogenic enzymes, including 3β-HSD, is associated with these changes in T production.

Ontology studies on testis have been performed to examine T production in relation to 3β-HSD activity. Immunohistochemistry studies have revealed that human Leydig cells express 3β-HSD as early as 18 wk gestation (210). During gestation in human, 3β-HSD expression in the testis parallels androgen production and is detected at the second and third trimesters. Eight-month-old and 11-yr-old boys showed no testicular 3β-HSD staining, and levels remained low until puberty (390). In the rodent, 3β-HSD protein was detected in fetal rat testis on d 14.5 post coitum (p.c.) (391).

Mouse type I/VI 3β-HSD is expressed in adult testis, and RT-PCR analysis showed that type I was expressed from embryonic d 13 until adulthood, whereas type VI was first detected at d 11 postnaturally and was the predominant isoform in the adult animal (280, 392). Clearly, expression of 3β-HSD during development is an indicator of testicular androgen production.

Adult Leydig cells arise postnatally and encompass three developmental stages: progenitor, immature, and adult cells (393). Rat testes of postnatal d 15 showed 3β-HSD localiza-
tion to the smooth ER of precursor Leydig cells and endothelial cells in the vicinity of 3β-HSD positive Leydig cells (394, 395). Large (12 μm) rat Leydig cells showed higher staining of 3β-HSD than smaller cells (8 μm) (396). Furthermore, cells from the rat H540 Leydig cell tumor line express 3β-HSD, P450c17, P450scc, P450arom, and 5α-reductase (397, 398). Other localization studies have examined 3β-HSD by immunocytochemistry in cynomolgus monkey testis, showing that 3β-HSD expression is seen in both Leydig and Sertoli cells (399). The immunoactive 3β-HSD was labeled in part of Leydig cells and in all Sertoli cells in neonatal, late infantile, pubertal, and adult testes, whereas only a few Leydig cells, but no Sertoli cells, were labeled in early infantile testes (400). Thus, the predominate site of 3β-HSD expression in the testis is in Leydig cells, but evidence exists for some localization in Sertoli cells, at least in some primate species.

2. LH. During development, testicular 3β-HSD expression appears to be uncoupled from LH regulation. T production is first detected in humans at 9 wk (383), yet LH receptors do not appear until 12 wk (277). Studies in normal mice have shown that LH levels can be seen at d 17 p.c., which is after induction of 3β-HSD in fetal Leydig cells. 3β-HSD expression was also shown to be normal and independent of circulating GnRH during development (401). In rats, 3β-HSD protein was detected in fetal testis on d 14.5 p.c., whereas LH receptor expression was seen on d 16.5 p.c., also suggesting an independent mechanism of 3β-HSD regulation in the fetal rat testis (391).

In adult rat testis, however, primary control of 3β-HSD expression occurs through the action of the LH receptor and the induction of the cAMP second messenger system. LH, forskolin, cAMP, and cholera toxin all induced 3β-HSD mRNA in adult rat Leydig cells (402, 403), whereas hCG and cAMP induced 3β-HSD activity in immature rat Leydig cells (404). hCG also induced 3β-HSD activity in adult rat testis (405, 406). However, desensitizing doses of hCG down-regulated 3β-HSD mRNA in adult rat Leydig cells, whereas it had no effect on 3β-HSD mRNA in pseudopregnant rat ovaries (407). Furthermore, K9 mouse Leydig cells respond to hCG with a 4-fold increase in 3β-HSD mRNA (408). LH secretion by the pituitary is therefore a critical control pathway in adult rodent testicular steroidogenesis, which is in contrast to fetal regulatory mechanisms.

3. Steroids. Studies have suggested that androgens can inhibit androgen production by the testis and that this repression could occur at the level of 3β-HSD regulation. Rats treated with hCG showed an increase in 3β-HSD activity, but R1881 (androgen agonist) decreased hCG induction, whereas cyproterone acetate (androgen antagonist) increased activity (405). Similarly, DHT and methyltrienolone (androgen agonist) inhibited 3β-HSD activity in adult rat Leydig cells (409), and T decreased 3β-HSD activity in Leydig cells from C57BL/6j mice (134, 410). Disruption of steroidogenesis with aminogluthethimide (AMG) treatment increased 3β-HSD and P450c17 mRNA levels, whereas treatment with T and cAMP reversed this effect (411). DEX (glucocorticoid agonist) addition in the presence of AMG reduced both basal and cAMP stimulatory effect in 3β-HSD mRNA, but not P450c17 in
mouse Leydig cells (411). AMG has also been shown to increase 3β-HSD mRNA in porcine Leydig cells; however, in contrast to mice this effect could not be rescued by T treatment (412). DEX increased P450 content and decreased 3β-HSD activity in rat Leydig cells, and older rats appeared more sensitive than younger rats (413). The data concerning down-regulation of 3β-HSD by androgens and up-regulation by glucocorticoids suggests that steroid regulation occurs in testicular Leydig cells and differs between species.

4. Stress. A study by Orr and Mann (414) showed that immobilization stress (IMO) of rats results in a reduction of serum androgen but not of serum LH concentrations. For example, 3 h of IMO to rats showed a 7-fold increase in serum corticosterone along with a reduction in serum T concentrations. Western blots of testicular fractions revealed reduced amounts of P450sc and 3β-HSD, but not P450c17 (415). Treatment with an opioid antagonist, naltrexone-methobromide, reversed this effect, suggesting that endogenous opioid peptides might play a role in regulating testicular steroidogenesis (416–418). Another study has suggested that IMO of rats results in the down-regulation of P450c17 and 3β-HSD and is mediated through nitric oxide (NO) synthase (419). Testicular injection of aminoguanidine hydrochloride (inducible NO synthase inhibitor) prevented the sustained, but not the acute, stress-induced decrease in serum T. In addition, aminoguanidine hydrochloride added in vivo and in vitro blocked the sustained decrease in 3β-HSD and P450c17 activities. These data seem to suggest that P450c17 and 3β-HSD are regulated in the testis independently of LH and that opioids and/or NO production might mediate this effect. In addition, the direct role of glucocorticoids in the modulation of testicular 3β-HSD transcription has yet to be examined. It should however be kept in mind that the effect of stress on serum LH concentrations varies according to the type of and length of stress.

5. Growth factors. Studies have been performed to determine whether 3β-HSD regulation in fetal testis might be mediated through growth factors. EGF, TGFβ, activin A, and GH have been shown to increase 3β-HSD activity. It has been demonstrated that EGF treatment of porcine Leydig cells increased 3β-HSD activity (420), whereas TGFβ and activin A treatment increased 3β-HSD activity in immature porcine Leydig cells (421). GH induced StAR and 3β-HSD mRNA expression as well as androgen production in progenitor Leydig cells isolated from 28-d-old rats, whereas cycloheximide treatment altered this 3β-HSD induction (422). FGF and platelet-derived growth factor have been shown to reduce 3β-HSD activity in immature rat Leydig cells. aFGF in immature rat Leydig cells decreased 3β-HSD activity (423), whereas insulin/IGF-1 treatment reversed the bFGF inhibitory effect in immature rat Leydig cells (423). Another report has shown that platelet-derived growth factor induces 3β-HSD activity induction by cAMP/HCG but not basal activity in cultured immature rat Leydig cells (424). Candidate growth factors might play a role in the regulation of 3β-HSD in the developing testis; however, the precise regulators of testicular 3β-HSD in vivo remain to be fully defined.

6. Other regulatory mechanisms. Other factors besides LH, steroids, stress, and growth factors have been tested for their ability to regulate 3β-HSD. Some of these include immune cytokines, neurotransmitter agonists, and oxidative metabolites. Amphetamine treatment of rat Leydig cells decreases 3β-HSD (425), whereas H2O2 decreased 3β-HSD activity in mouse MA-10 cells in a dose-dependent manner (426). Citrate removal from culture media of rat Leydig cells enhances LH-stimulated steroidogenesis, and this is independent of 3β-HSD, but may involve an increase of StAR (427). CSF-1 deficient mouse Leydig cells have reduced amounts of 3β-HSD, P450sc, P450c17, and 17β-HSD protein (428). TNFa and IL-1 decreased cAMP-stimulated 3β-HSD mRNA and protein in mouse Leydig cells, however only TNFa reduced basal levels of 3β-HSD and T output (429). Taken together, these studies suggest that a complex interplay of extrinsic ligands (i.e., LH) and intrinsic factors modulates androgen production by the testis.

D. Placenta

The placenta is responsible for the production of the majority of progestins and estrogens in primates during later stages of pregnancy. The 3β-HSD expressed in human placenta is the peripheral isofrom, type I 3β-HSD, and is under differential regulatory control than the adrenal/gonadal isoform, type II 3β-HSD as previously described. It should be noted that the catalytic efficiency of human type I 3β-HSD is 5.9-, 4.5-, and 2.8-fold higher than the catalytic efficiency (Vmax/Km) of the human type II 3β-HSD (adrenal/gonadal) isofrom using PREG, DHEA, and DHT, respectively, as substrates (27). The higher Km for type II 3β-HSD is correlated to the higher amounts of substrate produced in these tissues (430). A detailed understanding of the regulation of 3β-HSD in the placenta and other peripheral tissues must take into consideration the catalytic differences between the two isoforms present in humans.

1. Ontogeny and localization. It is generally considered that human 3β-HSD expression in the placenta is localized to syncytiotrophoblastic cells. 3β-HSD mRNA and protein were detected in uninnucleate cytotrophoblasts in chorion laeve similar to that in the syncytiot, but not in cytotrophoblast placenta. The expression of 3β-HSD was not induced either temporally or by cAMP in laeve cytotrophoblasts, whereas villous cytotrophoblasts spontaneously demonstrated progressive increases in 3β-HSD expression (161). Another study in humans has shown that 3β-HSD protein was localized to syncytiotrophoblast and intermediate trophoblast cells at both villous and extravillous sites, but not in cytotrophoblast cells from 6 wk gestation to term (431). Although several aspects of the baboon placenta are different from that of human, syncytiotrophoblast expression of 3β-HSD mRNA remained constant during all phases of gestation (432).

In contrast to primates, the mouse placenta is not of crucial importance in the de novo synthesis of steroids in the second half of pregnancy (347). However, recent reports have shown a role for the local production of steroids in the maternal decidua and trophoblast layers surrounding the endometrial...
cavity. Mouse type VI 3β-HSD is expressed in the uterine wall during decidualization (433). Expression is initially in the antimesometrial cells of the decidua on d 6.5–7.5 p.c. and then shifts to embryonal giant trophoblast cells. 3β-HSD VI then declines between d 10.4 and 14.5 p.c. (433). These data thus suggest that maternal expression of placental steroidogenic enzymes may play a determining role in maintenance of pregnancy and/or embryogenesis in a localized manner.

2. Regulation. Activation of PKA and PKC pathways in human placental in vivo models results in an increase in 3β-HSD activity. PMA and cAMP induces 3β-HSD mRNA in human JEG-3 choriocarcinoma cells; however, the phorbol ester response is mediated by PKC (159). A23187 increased basal 17β-HSD-I and 3β-HSD-I, but not P450sc in human JEG-3 cells. Furthermore, A23187 decreased cAMP stimulated type I 3β-HSD and P450sc, but synergistically activated type I 17β-HSD (434). Cross-talk between calcium flux/PKC and cAMP/PKA signaling pathways in the regulation of placental steroidogenesis is thus evident.

Growth factors have been tested for their ability to induce 3β-HSD in the human placenta. For example, insulin and IGF-I treatment of human cytotrophoblasts increased 3β-HSD activity (435). IGF-II also increased 3β-HSD activity in human placent al cytotrophoblasts, and this effect could be inhibited via cycloheximide or actinomycin D treatment (436). In addition, Morrish et al. (437) have examined the effects of cytotrophoblast differentiation on gene expression. EGF, colony-stimulating factor (CSF)-1, and granulocyte-macrophage CSF stimulate, whereas TGFβ inhibits human cytotrophoblast differentiation, and subtractive hybridization experiments between undifferentiated and differentiating cytotrophoblasts identified an increase in the hCG α-subunit, β1-glycoprotein, 3β-HSD, and plasminogen activator inhibitor-1 mRNAs during differentiation (437). These results suggest that growth factors involved in placental differentiation might play a role in the regulation of 3β-HSD and other factors. Although species- and placental-specific mechanisms are observed, similar regulatory pathways as those seen in other tissues exist for 3β-HSD expression in the placenta.

It is interesting that sex steroids have been shown to regulate 3β-HSD mRNA levels in trophoblast cells (438). Treatment of human trophoblast cell cultures with PROG and E2 led to increases in P450sc and 3β-HSD mRNA levels, but had no effects on 3β-HSD protein levels (438). The potential transcriptional regulation of the human type I 3β-HSD gene by PROG or E2 is interesting in light of the lack of palindromic steroid regulatory elements in the promoter and 5′-flanking sequence. In this regard, the human HSD3B1 gene may share some similar mechanisms with the HSD3B2 gene that has recently been shown to respond to PROG and glucocorticoids, although it is also lacking palindromic steroid regulatory elements (132).

The murine ortholog, type VI 3β-HSD, is the only isoform expressed in giant trophoblast cells during the first half of mouse pregnancy. Transfection studies in placental and non-placental cells identified a novel 66-bp trophoblast-specific enhancer element located between –2896 and –2831 of type VI 3β-HSD promoter (197). As revealed by DNase protection analysis, three trophoblast-specific binding sites, FPI, FPII, and FPIII, were identified. EMSAs with oligonucleotides representing the protected sequences, FPI and FPIII, and nuclear extracts isolated from human JEG-3 cells and from mouse trophoblast cells demonstrated the same binding pattern that was different from the binding pattern with mouse Leydig cell nuclear proteins. Additional assays identified AP-2γ and the homeodomain protein, Dlx-3, as the transcription factors that specifically bind to FPI and FPIII, respectively. Site-specific mutations in each of the binding sites eliminated enhancer activity, indicating that AP-2γ and Dlx-3, together with an additional transcription factor(s) that is conserved between humans and mice, are required for trophoblast-specific expression of type VI 3β-HSD (197).

E. Liver

Hepatic 3β-HSD expression is presumed to be important in the metabolism and inactivation of steroids. 3β-HSD has been purified from human liver (439, 440), and 3β-HSD activity in human liver microsomes was shown to be three times higher for the reduction of DHT to 3β-DIOL than the reverse reaction (441). The adult mouse liver expresses 3β-HSD types II, III, and IV, with type III predominating. Mouse type I 3β-HSD (analogous to human type II) is the predominant form in the fetal liver during development until postnatal d 1, when type III is induced. Mouse type I expression ends at postnatal d 20, while type V is detected at postnatal d 40 and is male specific, whereas type II is expressed at low levels throughout development. These data suggest that the mouse liver might play a key role during fetal development (95). Rat type III is expressed in the male rat liver and is a 3-KSR implicating its function in inactivating steroids in this tissue (75, 85, 442). Immunohistochemical studies localized 3β-HSD protein to the bile duct epithelium and microsomal fraction in the pig liver (98, 99).

Studies have also examined the regulation of 3β-HSD in the rodent liver. It appears that isoform-specific, sexually dimorphic regulation of 3β-HSD occurs in rats and mice. Castration of rats led to an 80% reduction in type III 3-KSR in rat liver after 15 d, and this effect could be rescued by the administration of DHT. Similarly, DHT increased levels in ovarietomized females to levels seen in the male (85). T increased, whereas E2 decreased type III 3-KSR in gonadectomized rats, but these effects were not seen in HYPOX animals. Also, PRL exerted a sex-specific blockade of T/E2 regulation in HYPOX females (85). A down-regulation of type III 3-KSR by GH was also observed in rats (85, 443). In the mouse liver, sexually dimorphic GH expression regulates many steroidogenic enzymes. GH expression in transgenic mice driven by the phosphoenolpyruvate-carboxykinase promoter down-regulates the 42-kDa male-specific isoform without affecting the 47-kDa nondimorphic isoform (444). These data suggest that circulating levels of steroids might affect regulation of 3β-HSD activity in the liver, principally through altering GH and PRL levels, and thereby resulting in feedback on steroid degradation.
**F. Breast**

Sex steroids are well recognized to play a predominant role in the regulation of cell growth and differentiation of normal mammary gland as well as in hormone-sensitive breast carcinomas. Estrogens stimulate cell growth of hormone-sensitive breast cancer cells (445–448), whereas androgens exert an antiproliferative action in breast cancer cells (12, 448–450). The role of estrogen formation in peripheral intracellular tissues is well illustrated in women by the important benefits on breast cancer observed in postmenopausal women treated by a series of aromatase inhibitors (451). Moreover, the recent observation that postmenopausal women who received the antiestrogen raloxifene for only 3 yr led to a 76% decrease in the incidence of breast cancer (452) is also consistent with the role of extra ovarian estrogens in the development and growth of breast cancer. 3β-HSD activity was detected in breast tissue in humans as early as 1990 (453), and 3β-HSD activity was demonstrated in human adipose stromal tissue (454). Expression in breast cancer tissue has also been investigated. Stage II/III infiltrating ductal primary breast tumors demonstrated 3β-HSD activity (455), and 3β-HSD protein was seen in 36% of breast carcinoma samples tested (456). Similarly, 3β-HSD activity was detected in ZR-75-1 breast cancer cells (457).

Both endocrine and paracrine influences on the proliferation of human breast cancer cells are well recognized, thus supporting the suggestion that breast tumor growth is modulated by the hormonal environment (458, 459). The presence in breast tumors of considerable numbers of tumor-associated macrophages and tumor-infiltrating lymphocytes secreting a wide spectrum of cytokines also suggests a key role for these factors in neoplastic cell activity (460, 461). Moreover, it has been observed that Natural Killer cells isolated directly from ductal invasive breast tumors secrete increased amounts of interferon-γ and IL-4 (462). The rationale to investigate the potential action of IL-4 and IL-13 in breast cancer cells pertains to the recent demonstration of high-affinity IL-4 receptors in human breast carcinomas (463), the inhibitory effect of IL-4 and IL-13 on the estrogen-induced breast cancer cell proliferation (171, 183, 464, 465) and their potent stimulatory effect on GCDP-15/PIP gene expression (183), as well as on spermidine transport in ZR-75-1 cells (466). Furthermore, it has been reported that some cytokines can regulate the expression of several enzymes involved in sex steroid formation and inactivation in breast cells. For example, IL-6 regulates the expression of 17β-HSD/KSR (171, 467), estrone sulfatase (468), and P450 aromatase (15, 126), whereas IL-4 regulates 17β-HSD/KSR (171) activity as well as 3β-HSD type 1 activity (51, 52, 171).

Under basal growth condition, there is no detectable 3β-HSD activity in ZR-75-1 cells; however, incubation with IL-4 or IL-13 caused a potent up-regulation of 3β-HSD activity (51). It should be noted that IL-1α, IL-2, IL-3, IL-6, IL-8, IL-10, interferon-γ, and EGF all failed to induce 3β-HSD activity in ZR-75-1 cells. In the ER− MDA-MB-231 cells, IL-4 increased this enzymatic activity by 3.3-fold as measured by the conversion of [3H]DHEA into both [3H]4-DIONE and [3H]A-DIONE, thus indicating that the IL-4 action on 3β-HSD activity was not restricted to ER− ZR-75-1 and T-47D human breast cancer cells. Furthermore, the 5.5-fold increase in 3β-HSD activity after exposure to IL-4 in normal human mammary epithelial cell in primary culture suggests the physiological relevance of our finding.

As shown in Fig. 14, IGF-I and insulin increase the stimulatory effect of IL-4 on 3β-HSD activity. The stimulatory effect of IGF-I and insulin on the IL-4-induced 3β-HSD activity was exerted at EC50 values of 0.8 and 3 nm, respectively, thus providing the first evidence of potentiation of an IL-4 response by IGF-I and/or insulin. The relevance of this finding also pertains to the observation that most invasive breast tumors appear to express IGF-I receptor, and their growth is stimulated in vitro in response to exogenous IGF-I (469). Furthermore, it is of interest to note that the amplitude of the effect of insulin or IGF-I on IL-4-induced 3β-HSD activity was more striking in the presence of submaximal concentrations of the cytokine. It is thus tempting to speculate that the action of these growth factors could be significant under physiological conditions when IL-4 is present at low concentrations.

The similar effects exerted by IL-4 and IL-13 are explained by the fact that their receptors share at least one subunit (470, 471). One type of IL-4R is composed of the IL-4Ro chain and the common γ chain from the IL-2 receptor, whereas the IL-13 receptor a1 chain can heterodimerize with the IL-4Ra chain to form receptors for both IL-4 and IL-13 (472).

Because aromatase is expressed in a large proportion of human breast carcinomas (15, 473, 474), induction of 3β-HSD activity by IL-4 and IL-13 could markedly increase the formation of estrogen precursors, namely Δ5-DIONE and T, and therefore may well have a significant impact on the estrogen synthesis in breast tumors. However, it should be taken into consideration that androgens are well recognized to exert an antiproliferative action in breast cancer cells (446, 447). Thus, the IL-4-induced 3β-HSD activity would first increase the intracellular levels of T that in return would inhibit breast cancer cell growth. In support of this hypothesis, DHEA exerts an inhibitory effect on the development of ZR-75-1 human breast cancer cell xenografts in ovariectomized nude mice (450). Moreover, DHEA exerts a potent inhibitory effect on the development of dimethylbenzanthracene-induced mammary carcinoma in the rat (475). Finally, DHEA exerts an almost exclusive androgenic effect in the rat mammary gland (476).

Δ5-DIOL is well recognized to exert an estrogenic action at physiological concentrations due to its relatively high affinity for estrogen receptors, while it also possesses a lower affinity for the androgen receptor (446, 448, 449, 477, 478). Thus, as demonstrated by an experiment using both estrogen- and androgen-sensitive reporter constructs, the induction of type I 3β-HSD expression by IL-4 may indeed modulate the balance between estrogenic and androgenic biological responses when incubating ZR-75-1 cells with Δ5-DIOL (51). Our findings thus strongly support the relevance of the action of IL-4 and IL-13 in the biosynthesis of active sex steroids from adrenal precursors (DHEA and Δ5-DIOL) in both normal and tumoral human breast tissues.

**G. Prostate**

In men, the importance of intracrine formation of active androgens is in concordance with the observation that after
elimination of testicular androgens by medical or surgical castration, the intraprostatic concentration of DHT remains at approximately 40% of that measured in the prostate in intact 65-yr-old men, thus leaving important amounts of free androgens to continue stimulating growth of the prostate cancer (13, 479, 480). Indeed, an important source of androgens in the human prostate is androgens synthesized locally by the conversion of adrenal-derived DHEA and DHEA-S by 3β-HSD and 17β-HSD type V into Δ5-DIONE and T, respectively.

It has been shown that 3β-HSD expression colocalizes by immunocytochemistry and in situ hybridization with 17β-HSD type V in the glandular epithelium of the prostate with the highest levels seen in basal cells relative to luminal cells (481). Other studies have found 3β-HSD in human hyperplastic prostate tissue (482, 483). These studies demonstrate the capacity of the human prostate to locally produce androgens, which increase the hypertrophic potential of the organ.

More recently, IL-4 and IL-13 were shown to induce 3β-HSD activity and mRNA levels in normal human prostate epithelial cells (PrEC) (52). The 3β-HSD activity was not detectable in PrEC under basal growth conditions, whereas incubation with IL-4 or IL-13 induced a potent up-regulation in 3β-HSD activity observed at EC50 values of 20 and 170, respectively. Moreover, IL-4 induced 3β-HSD gene expression as early as 3 h after exposure (52), whereas the 3β-HSD activity was detectable after a short incubation period of 8 h with IL-4 and continued to increase for at least 48 h, thus showing a rapid induction of 3β-HSD activity by IL-4. Thus, the induction of 3β-HSD activity by IL-4 and IL-13 would markedly increase the formation, from DHEA, of the type V 17β-HSD/KSR substrates Δ5-DIONE and A-DIONE, which would lead to the synthesis of T and DHT, respectively. This may well have a significant impact on the development of prostate cancer because the effect of prolonged presence of androgens that stimulate prostate cancer growth is well established (13, 484, 485). The relevance of the IL-4 action in prostate cells also pertains to the observation that in an immortalized human prostate cell line derived from primary cultures, the gene expression of the tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 was regulated by IL-4 (486). It was suggested that IL-4 might control the molar ratio of tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 to influence the level of protease activity and perhaps the invasive behavior of malignant cells in vivo.

In the general population, we observe an interindividual variability in the susceptibility to cancer; however, little is known about the underlying genes contributing to this variability. Although a number of rare highly penetrant loci contribute to the Mendelian inheritance of prostate cancer as recently described (487), some of the familial risks may be due to shared environment and more specifically to common low-penetration genetic variants, which alter predisposition to prostate cancer. It is not surprising that analyses of genes encoding key proteins involved in androgen biosynthesis and action, led to the observation of a significant association between common genetic variants and a susceptibility to prostate cancer (488–491). Such analyses provided some understanding of how common low-penetration polymorphisms present in a number of these candidate genes were involved in prostate cancer onset, progression, and response to treatment for the disease. To evaluate the possible role of HSD3B genes in prostate cancer susceptibility, a recent study used a panel of DNA samples collected from 96 men with or without prostate cancer for sequence variants in the putative promoter region, exons, exon-intron junctions, and 3′-untranslated region of HSD3B1 and HSD3B2 genes by direct sequencing (492). Four of the 11 single nucleotide polymorphisms (SNPs) were informative. These four SNPs were further genotyped in a total of 159 hereditary prostate cancer probands, 245 sporadic prostate cancer cases, and 222 unaffected controls. Although a weak association between prostate cancer risk and a missense SNP (B1-N367T) was found, stronger evidence for association was found when the joint effect of the two genes was considered. Indeed, men with the variant genotypes at either B1-N367T or B2-c7519g had a relative risk of 1.76 (95% confidence interval, 1.21–2.57; P = 0.003) to develop prostate cancer compared with men who were homozygous wild type at both genes. The risk for the hereditary type of prostate cancer was stronger, with a relative risk of 2.17 (95% confidence interval, 1.29–3.65; P = 0.003). Most importantly, the subset of hereditary prostate cancer probands, whose families provided evidence for linkage at 1p13, predominantly contributed to the observed association (492). Additional studies are warranted to confirm these findings.

The rat provides a useful model for the study of prostate steroid production. 3β-HSD activity was seen in the rat ventral prostate (493) with higher activity in epithelial vs. stromal cells (494). In addition, the 5α-reductase inhibitor, 4MA, was shown to decrease 3β-HSD activity in the prostate of rats (495), thus suggesting that androgens may play a role in the regulation of prostatic steroidogenesis; however, more work needs to be done to verify these effects.

**H. Skin**

Sharp et al. (496) have shown that 3β-HSD activity in human fetal skin is low at 12 wk, whereas 28-, 38-, and 41-wk human fetal skin samples showed high 3β-HSD activity. It was also observed that 90% of 3β-HSD activity from human fetuses was found to be in sebaceous glands (497). Thereafter, it has been demonstrated that human 3β-HSD is found in sebaceous gland ducts with highest expression in fully differentiated ducts (53, 498, 499). 3β-HSD expression in sebaceous glands provides the ability of glands to control local concentrations of DHEA and other steroids in the skin.

It has been demonstrated recently that IL-4 and IL-13 caused a rapid induction of 3β-HSD activity in HaCaT human immortalized keratinocytes (Fig. 13) (52, 500). The physiological relevance of this finding is well supported by the observations that: 1) human skin can convert DHEA into T (501); 2) DHEA can stimulate sebaceous gland secretion (502, 503); 3) the in vivo sebum secretion rate in humans is closely correlated with 3β-HSD activity (504); and 4) 3β-HSD type 1 gene is expressed in human skin (27, 53). The relevance of this finding also pertains to the observation that IL-4 and IL-13 stimulate IL-6 expression in normal keratinocytes and ker-
atinocyte cell lines of human origin (505), and that IL-4 induces proliferation of normal human keratinocytes, which is associated with c-myc gene expression (506). Therefore, it is likely that these cytokines play a role in the regulation of inflammation at both systemic and local levels. Finally, human keratinocytes constitutively express IL-4R, and its expression is also increased in some epidermal proliferative diseases (507, 508), whereas IL-4 can be detected in atopic dermatitis and during the elicitation phase of contact sensitivity (507, 509). Thus, it would be relevant to investigate the potential effect of androgens or estrogens in those pathologies as well as in the physiological functions of keratinocytes.

In rats, type IV is the major isoform in skin (83) with much lower expression of types II and I, whereas the only 3β-HSD transcript found in mouse skin is the type VI (91, 92). HYPOX rats showed an increase of 3β-HSD activity in female animals and a decrease in males. Moreover, treatment of HYPOX animals with l-T₄ demonstrated elevated 3β-HSD mRNA in a male-specific manner, corticosterone showed a decrease in mRNA in both sexes, and elevated PRL levels caused a stimulation of type IV in both sexes (510).

I. Brain
Steroid hormones exert important functions in the control of growth, maturation, and differentiation of the central and peripheral nervous systems. These actions have long been attributed exclusively to steroid hormones secreted by adrenal glands and gonads. However, during the past decade, it has been demonstrated that both neurons and glial cells are capable of synthesizing bioactive steroids, now called neurosteroids, which also participate in the control of various functions in the central nervous system (CNS). Several recent studies have made possible the establishment of the neuroanatomical distribution of key enzymes (see Ref. 511 for a review). Levels of PROG are higher in the peripheral nerves of rats and mice than in plasma, and these levels persist after removal of steroidogenic endocrine glands (512). It should be noted that examination of PROG production must be coupled with relative levels of PROG metabolites to fully understand the neurosteroid milieu present in specific regions of the brain.

The presence of 3β-HSD activity in the CNS was first demonstrated in homogenates of rat amygdala and septum (513), whereas the first immunohistochemical localization of 3β-HSD in the CNS was performed in the European green frog Rana ridibunda (514). Multiple 3β-HSD localization studies in rats have characterized expression at the gross neuroanatomical level. For instance, P450sc and 3β-HSD are expressed as demonstrated by in situ hybridization in the hippocampus, dentate gyrus, cerebellar granular layer, olfactory bulb, and Purkinje cells of the rat brain (515). Other studies showed by RT-PCR that 3β-HSD expression occurs in the cerebellum and cerebrum of the rat brain (516). 3β-HSD mRNA was also localized to the olfactory bulb, striatum, cortex, thalamus, hypothalamus, septum, habenula, hippocampus, and cerebellum in the rat brain. The cerebellum showed the highest levels of expression of a 1.8-kb transcript (517). Rat 3β-HSD mRNA was also localized in the medulla ventrally and laterally bordering the fourth ventricle (518). In situ studies showed 3β-HSD expression in neonatal rat Purkinje cells and external granule cells throughout the cerebellum at 10 d of age (519). In the peripheral nervous system, rat 3β-HSD was localized in the sciatic nerve (520, 521) as well as in dorsal root ganglion, where its expression is increased after peripheral nerve injury (522). Although expression patterns of steroid metabolizing enzymes in the brain are emerging, the functional implications of local steroid production are only beginning to be examined.

Other studies have demonstrated 3β-HSD expression in cell culture. Rat Schwann cells and sensory neurons from the embryonic dorsal root ganglia express 3β-HSD protein and activity (523). RT-PCR studies have shown that rat cerebellar glial and cerebellar granule primary cell cultures expressed 3β-HSD and P450sc (524). Rat astrocytes express 3β-HSD activity (525, 526), whereas 3β-HSD, 3α-HSD, and 5α-reductase activities can be detected in fetal rat brain cells in culture (527). 3β-HSD was increased 10-fold in rat Schwann cell/neuronal cocultures, suggesting that 3β-HSD regulation in isolated cells differs from that seen in vivo. Moreover, 3β-HSD and P450sc mRNAs were induced during the differentiation of O-2A precursor cells to oligodendrocytes (528). Astrocytes appear to be the most steroidogenically active cells in the brain as determined from cells isolated from the neonatal rat cerebral cortex (529). Finally, it has been reported that in frog hypothalamic explants that the endogenous triakontatetraneuropeptide stimulates 3β-HSD activity. This effect was mimicked by a peripheral benzodiazepine receptor (PBR) agonist and inhibited by the PBR antagonist PK11195, whereas the central-type PBR antagonist, flumazenil, did not affect the triakontatetraneuropeptide-evoked neurosteroid secretion (530). These cell culture models therefore provide systems in which regulatory factors for the control of neurosteroidogenesis can be identified.

J. Other expression sites
The importance of local steroid production by peripheral isoforms of 3β-HSD is easily envisioned. However, far too little is known about the regulation of 3β-HSD in additional peripheral tissues, namely, kidney (88), vas deferens (531), bone (532, 533), cardiovascular tissues (534, 535), adipose tissue (545), etc. Nevertheless, as one example supporting the key role of 3β-HSD activity in peripheral tissues, it has been recently observed that 3β-HSD is expressed in mouse megakaryocytes in which it is essential for the initiation of proplatelet formation. A recent study showed that 3β-HSD expression is up-regulated by the megakaryocyte/erythrocyte specific transcription factor P45 NF-E2 (536). This up-regulation induces biosynthesis of estrogens in matured megakaryocytes, which in turn, by autocrine action on the estrogen receptor, triggers proplatelet formation. Because platelet transfusions are presently the only established therapy for preventing bleeding complications in severely thrombocytopenic individuals, these results provide an interesting venue for the development of an effective treatment of thrombocytopenia (536). The molecular mechanisms involved in the regulation of 3β-HSD in classical steroidogenic tissue appears to have little overlap due to the lack of control by trophic factors in these tissues, but cytokines, GH, and
PRL are good candidates to first investigate. Thus, the mapping of hormones and transcription factors involved in the control of each of these tissues points to a potentially rich source of regulatory pathways involved in peripheral steroidogenesis that have yet to be elucidated. An understanding of these pathways will allow for controlled modulation of steroidogenesis and its effects in these tissues.

VII. Molecular Genetics of Human 3β-HSD Deficiency

Classical 3β-HSD deficiency is a rare form of congenital adrenal hyperplasia (CAH) accounting for about 1–10% of cases of CAH (537, 538). The salt-losing forms of CAH are a group of life-threatening diseases that require prompt recognition and treatment. Indeed, autosomal recessive mutations in the CYP21, CYP17, CYP11B1, and HSD3B2 genes that encode steroidogenic enzymes, in addition to mutations in the gene encoding the intracellular cholesterol transport protein, StAR, can cause CAH, each resulting in different biochemical consequences and clinical features (277, 539–541). These five biochemical defects impair cortisol secretion, which results in compensatory hypersecretion of ACTH and consequent hyperplasia of the adrenal cortex. However, only deficiencies in 21-hydroxylase (CYP21) and 11β-hydroxylase (CYP11B1) predominantly result in virilizing disorders. Indeed, in patients with the classical form of these two defects, the most noticeable abnormality in the sexual phenotype is the masculinization of the female fetus due to oversynthesis of adrenal DHEA and DHEA-S, whereas in comparison, affected males do not have ambiguous genitalia. In contrast to these two most frequent causes of CAH, which are exclusive adrenal defects, 3β-HSD, P450c17, and StAR deficiencies impair steroidogenesis in both the adrenals and the gonads, resulting in the diminished formation of sex steroids in addition to cortisol and aldosterone.

A. Clinical features

Since the first reports by Bongiovanni (542, 543), many patients of both sexes have been described with various heterogeneity upon clinical presentation (Table 2). Classical 3β-HSD deficiency results from mutations in the HSD3B2 gene, whereas the HSD3B1 gene in these patients is normal (56–58, 539, 544–547). The classical form of 3β-HSD deficiency can be divided, depending upon the severity of the salt-wasting associated with the disorder, into the salt-wasting or non-salt-wasting forms. There is no correlation between the impairment in male sexual differentiation and salt wasting.

Male individuals suffering from classical 3β-HSD deficiency present with either perineal hypospadia or penoscrotal hypospadia as shown in Table 2. On the other hand, complete or partial inhibition of 3β-HSD activity in the adrenals and ovaries was not accompanied by a noticeable alteration in the differentiation of the external genitalia of female patients, as indicated by the absence of ambiguous external genitalia. The reason for this striking difference in phenotype between the male and female individuals is that deficiency of 3β-HSD in the fetal testis results in a lowering of the levels of T below the levels required for the normal development of external genitalia (56–58, 539, 544–547). However, males affected with pseudohermaphroditism and complete or partial 3β-HSD deficiency have intact Wolffian duct structures, including vas deferens. This is also the case in 17β-HSD type III deficiency as well as 5α-reductase type II deficiency, which is consistent with the hypothesis that a major effect of 3β-HSD deficiency is to reduce the formation of DHT below the level required for normal development of external genitalia (545). However, it is important to keep in mind that the decreased DHT results only from reduced T, knowing that the 5α-reductase activity is intact in these patients.

The salt-losing form of classical 3β-HSD deficiency is usually diagnosed during the first few months of life due to insufficient biosynthesis of aldosterone and consequent salt loss, which may be fatal if not diagnosed and treated early (542, 547, 549–554). In contrast, the non-salt-losing form of 3β-HSD deficiency may be diagnosed either at a young age in the presence of indicating factors, such as a family history of death during early infancy (555), perineal hypospadia in male newborns (556, 557), or failure to gain weight (553), or the diagnosis may be made at a later date (554, 558–560). It would be expected that death in early infancy is more indicative of salt loss; however, the family history of a patient diagnosed with the non-salt-losing form of 3β-HSD deficiency indicated that four of seven children died in early infancy (554). Because sexual differentiation is normal in female newborns affected by non-salt-losing 3β-HSD deficiency, the correct diagnosis is delayed until adrenarche (559, 561) or puberty (560) (Table 2).

Interestingly, the patient recently reported by Van Vliet and colleagues (561) underwent progressive feminization starting between 8 and 9 yr of age and was the first female with severe salt-wasting 3β-HSD deficiency reported to have progressive breast development, regular menses, and evidence of PROG secretion. This patient is homozygous for the A10E mutation that results in the complete loss of type II 3β-HSD activity (561). One possible explanation for the development of breasts and the endometrium in the patient reported by Van Vliet is that there is local conversion of inactive adrenal precursors into estrogens. On the other hand, it is possible that, as suggested for the tests (562), pubertal levels of gonadotropins may induce sufficient ovarian 3β-HSD activity by increasing the normally low levels of type I 3β-HSD, thereby allowing significant ovarian production of E2. The rise in the patient’s plasma PROG levels favors the latter hypothesis and raises the possibility that this individual may be fertile (561). It should be noted that a male affected with proven severe 3β-HSD deficiency has in fact fathered children (56, 549). These recent findings further illustrate that genotype may not always predict the phenotype in patients with this disorder (548).

B. Biological diagnosis

The basal plasma levels of Δ⁵-3β-hydroxy steroids such as PREG, 17OHPREG, and DHEA are elevated in affected individuals. An elevated ratio of Δ⁵/Δ¹-steroids is considered to be the best biological parameter for the diagnosis of 3β-HSD deficiency (560, 563). The best criteria for the correct
<table>
<thead>
<tr>
<th>Family Origin</th>
<th>Patient</th>
<th>Karyotype</th>
<th>Age at diagnosis</th>
<th>Phenotype</th>
<th>Salt-wasting</th>
<th>Mutant alleles</th>
<th>Apparent activity in intact cells</th>
<th>Evidence of protein instability</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homozygotes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pakistani</td>
<td>1</td>
<td>46 XY</td>
<td>Birth</td>
<td>Hypospadias, hyperpigmented and bifid scrotum</td>
<td>No (normal renin activity)</td>
<td>L6F /L6F</td>
<td>54.75 ± 8%</td>
<td>No</td>
<td>580</td>
</tr>
<tr>
<td>French-Canadian</td>
<td>2</td>
<td>46 XX</td>
<td>3 wk</td>
<td>Normal genitalia, breast development and menses at puberty</td>
<td>Yes</td>
<td>A10E/A10E</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>561</td>
</tr>
<tr>
<td>French-Canadian</td>
<td>3</td>
<td>46 XY</td>
<td>13 d</td>
<td>Ambiguous genitalia, palpable testes in bifid scrotum</td>
<td>Yes</td>
<td>A10E/A10E</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>561</td>
</tr>
<tr>
<td>Egyptian</td>
<td>4</td>
<td>46 XY</td>
<td>6 months</td>
<td>Perineocrotal hypospadias</td>
<td>No (normal renin activity)</td>
<td>A10V/A10V</td>
<td>29.1 ± 0.6%</td>
<td>No</td>
<td>59</td>
</tr>
<tr>
<td>Algerian</td>
<td>5</td>
<td>46 XY</td>
<td>Birth</td>
<td>Perineocrotal hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>G15D/G15D</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>555, 582</td>
</tr>
<tr>
<td>Brazilian</td>
<td>6</td>
<td>46 XX</td>
<td>31 yr</td>
<td>Clinically normal</td>
<td>No (normal renin activity)</td>
<td>A82T/A82T</td>
<td>7.6 ± 0.5%</td>
<td>No</td>
<td>59, 559, 577</td>
</tr>
<tr>
<td>Brazilian</td>
<td>7</td>
<td>46 XX</td>
<td>Birth</td>
<td>Ambiguous genitalia</td>
<td>No (normal renin activity)</td>
<td>A82T/A82T</td>
<td>7.6 ± 0.5%</td>
<td>No</td>
<td>59, 577</td>
</tr>
<tr>
<td>French</td>
<td>8</td>
<td>46 XY</td>
<td>20 months</td>
<td>Perineal hypospadias</td>
<td>No (elevated renin activity)</td>
<td>N100SN100S</td>
<td>2.8 ± 0.07%</td>
<td>No</td>
<td>553, 575</td>
</tr>
<tr>
<td>Chilian</td>
<td>9</td>
<td>46 XX</td>
<td>Birth</td>
<td>Hyperpigmentation, normal genitalia</td>
<td>Yes</td>
<td>E135X/E135X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>594</td>
</tr>
<tr>
<td>Swiss</td>
<td>10</td>
<td>46 XX</td>
<td>14.7 yr</td>
<td>Lack of spontaneous breast development</td>
<td>Yes</td>
<td>W171X/W171X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>56, 550, 578</td>
</tr>
<tr>
<td>Swiss</td>
<td>11</td>
<td>46 XX</td>
<td>?</td>
<td>Lack of spontaneous breast development</td>
<td>Yes</td>
<td>W171X/W171X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>56, 595</td>
</tr>
<tr>
<td>Scottish</td>
<td>12</td>
<td>46 XY</td>
<td>5 yr</td>
<td>Perineal hypospadias</td>
<td>No (normal renin activity)</td>
<td>L173W/L173R</td>
<td>52.8 ± 0.6%</td>
<td>No</td>
<td>59, 545</td>
</tr>
<tr>
<td>Japanese</td>
<td>13</td>
<td>46 XY</td>
<td>3 months</td>
<td>Hyperpigmentation, severe hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>L205P/L205P</td>
<td>No detectable activity</td>
<td>No</td>
<td>59, 596</td>
</tr>
<tr>
<td>Algerian</td>
<td>14</td>
<td>46 XX</td>
<td>11 months</td>
<td>Hyperpigmentation, mild clitoromegaly</td>
<td>Yes</td>
<td>P222Q/P222Q</td>
<td>No detectable activity</td>
<td>No</td>
<td>59</td>
</tr>
<tr>
<td>Algerian</td>
<td>15</td>
<td>46 XX</td>
<td>1 month</td>
<td>Mild clitoromegaly, first diagnosis 21-hydroxylase deficiency</td>
<td>Yes</td>
<td>P222T/P222T</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>597</td>
</tr>
<tr>
<td>Turkish</td>
<td>16</td>
<td>46 XY</td>
<td>4 yr</td>
<td>Scrotal hypospadias, bifid scrotum</td>
<td>No (normal renin activity)</td>
<td>A245F/A245F</td>
<td>35.4 ± 0.2%</td>
<td>Yes</td>
<td>57, 554</td>
</tr>
<tr>
<td>Japanese</td>
<td>17</td>
<td>46 XY</td>
<td>N/A</td>
<td>Pigmentation, hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>R249X/R249X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>598</td>
</tr>
<tr>
<td>Japanese</td>
<td>18</td>
<td>46 XY</td>
<td>N/A</td>
<td>Pigmentation, mild clitoromegaly</td>
<td>Yes</td>
<td>R249X/R249X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>598</td>
</tr>
<tr>
<td>Japanese</td>
<td>19</td>
<td>46 XY</td>
<td>Birth</td>
<td>Hypospadias with microopen and bifid scrotum</td>
<td>Yes</td>
<td>R249X/R249X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>562, 599</td>
</tr>
<tr>
<td>Japanese</td>
<td>20</td>
<td>46 XY</td>
<td>N/A</td>
<td>Pigmentation, hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>T259R/T259R</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>59, 598</td>
</tr>
<tr>
<td>Taiwanese</td>
<td>21</td>
<td>46 XY</td>
<td>Birth</td>
<td>Hypospadias, micropenis, palpable testes in scrotum</td>
<td>Yes</td>
<td>T259M/T259M</td>
<td>Very weak activity</td>
<td>Yes</td>
<td>59, 580</td>
</tr>
<tr>
<td>Brazilian</td>
<td>22</td>
<td>46 XX</td>
<td>31 yr</td>
<td>Clitoromegaly, severe virilization</td>
<td>No (normal renin activity)</td>
<td>T259M/T259M</td>
<td>Very weak activity</td>
<td>Yes</td>
<td>59, 600</td>
</tr>
<tr>
<td>Brazilian</td>
<td>23</td>
<td>46 XY</td>
<td>7.8 yr</td>
<td>Mild clitoromegaly</td>
<td>No (normal renin activity)</td>
<td>T259M/T259M</td>
<td>Very weak activity</td>
<td>Yes</td>
<td>59, 594</td>
</tr>
<tr>
<td>Sri-Lankan</td>
<td>24</td>
<td>46 XY</td>
<td>Birth</td>
<td>Perineal hypospadias with micropenis, palpable testes in scrotum</td>
<td>Yes</td>
<td>687del27/687del27</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>59</td>
</tr>
<tr>
<td>Sri-Lankan</td>
<td>25</td>
<td>46 XY</td>
<td>Birth</td>
<td>Perineal hypospadias</td>
<td>Yes</td>
<td>687del27/687del27</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>59</td>
</tr>
<tr>
<td>Afghan/Pakistani</td>
<td>26</td>
<td>46 XY</td>
<td>2 wk</td>
<td>Perineal hypospadias</td>
<td>Yes</td>
<td>818 del AA/818 del AA</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>601</td>
</tr>
<tr>
<td>Afghan/Pakistani</td>
<td>27</td>
<td>46 XY</td>
<td>Birth</td>
<td>Ambiguous genitalia, hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>818 del AA/818 del AA</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>601</td>
</tr>
<tr>
<td>Afghan/Pakistani</td>
<td>28</td>
<td>46 XY</td>
<td>Birth</td>
<td>Hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>818 del AA/818 del AA</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>601</td>
</tr>
<tr>
<td>Compound heterozygotes</td>
<td>Ethnic origin</td>
<td>Age</td>
<td>Sex</td>
<td>Phenotype</td>
<td>Mutation</td>
<td>Activity</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------</td>
<td>-----</td>
<td>-----</td>
<td>-----------</td>
<td>----------</td>
<td>----------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 English 36 46 XY 9 yr</td>
<td>English</td>
<td>9 yr</td>
<td>XY</td>
<td>Perineal hypospadias with normal testes, initial diagnosis of 21-hydroxylase deficiency (elevated renin activity)</td>
<td>No</td>
<td>N100S</td>
<td>1.3%</td>
<td>No</td>
<td>575, 602, 603</td>
</tr>
<tr>
<td>30 Spanish/Portuguese 38 46 XY Birth</td>
<td>Spanish/Portuguese</td>
<td>Birth</td>
<td>XY</td>
<td>Perineal hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>L108W</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>553, 604, 605</td>
</tr>
<tr>
<td>31 N/A 39 46 XX 43 months</td>
<td>N/A</td>
<td>43 months</td>
<td>XY</td>
<td>Minor virilization of external genitalia, premature pubarche, accelerated growth</td>
<td>No</td>
<td>S125G</td>
<td>N/A</td>
<td>N/A</td>
<td>606</td>
</tr>
<tr>
<td>32 American 41 46 XX 18 yr</td>
<td>American</td>
<td>18 yr</td>
<td>XX</td>
<td>Premature pubarche, acne, mild clitoromegaly</td>
<td>No (normal renin activity)</td>
<td>P155L</td>
<td>No detectable activity</td>
<td>No</td>
<td>59, 555</td>
</tr>
<tr>
<td>33 Brazilian 43 46 XX 6.7 yr</td>
<td>Brazilian</td>
<td>6.7 yr</td>
<td>XX</td>
<td>Normal genitalia, premature pubarche</td>
<td>No (elevated renin activity)</td>
<td>G129R</td>
<td>11.7 ± 0.1%</td>
<td>No</td>
<td>558, 574, 607</td>
</tr>
<tr>
<td>34 American 45 46 XY 1 wk</td>
<td>American</td>
<td>1 wk</td>
<td>XY</td>
<td>Perineal hypospadias, bifid scrotum</td>
<td>Yes</td>
<td>E142K</td>
<td>No detectable activity</td>
<td>No</td>
<td>57, 560</td>
</tr>
<tr>
<td>35 Caucasian 46 46 XX 7.3 yr</td>
<td>Caucasian</td>
<td>7.3 yr</td>
<td>XX</td>
<td>Normal genitalia, premature pubarche at 5.5 yr, acne</td>
<td>No</td>
<td>G294V</td>
<td>20.5 ± 4.6%</td>
<td>No</td>
<td>59, 555</td>
</tr>
<tr>
<td>36 French 47 46 XY Birth</td>
<td>French</td>
<td>Birth</td>
<td>XY</td>
<td>Perineal hypospadias, palpable testes in scrotum</td>
<td>No (elevated renin activity)</td>
<td>X373C</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>597</td>
</tr>
<tr>
<td>37 American 49 46 XY 34 yr</td>
<td>American</td>
<td>34 yr</td>
<td>XX</td>
<td>Hypospadias (1 month), gynecomastia (13 yr)</td>
<td>Yes</td>
<td>W171X</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>56, 608</td>
</tr>
<tr>
<td>38 French 50 46 XY Birth</td>
<td>French</td>
<td>Birth</td>
<td>XY</td>
<td>Perineal hypospadias with micropenis, no palpable testes</td>
<td>No (normal renin activity)</td>
<td>558insC</td>
<td>No predicted activity</td>
<td>100%</td>
<td>No</td>
</tr>
<tr>
<td>39 Dutch 51 46 XY Birth</td>
<td>Dutch</td>
<td>Birth</td>
<td>XY</td>
<td>Urethral diverticula and hypospadias</td>
<td>Yes</td>
<td>867delG</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>57, 609, 610</td>
</tr>
<tr>
<td>40 French 52 46 XY Birth</td>
<td>French</td>
<td>Birth</td>
<td>XY</td>
<td>Perineal hypospadias, palpable testes in scrotum</td>
<td>Yes</td>
<td>Y253H</td>
<td>No detectable activity</td>
<td>Yes</td>
<td>59</td>
</tr>
<tr>
<td>41 Pakistani 53 46 XX Birth</td>
<td>Pakistani</td>
<td>Birth</td>
<td>XX</td>
<td>Normal genitalia</td>
<td>Yes</td>
<td>867delG</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>611</td>
</tr>
<tr>
<td>42 American 55 46 XX Puberty</td>
<td>American</td>
<td>Puberty</td>
<td>XX</td>
<td>Primary amenorrhea, slight clitoromegaly, moderate hirsutism, enlarged polycystic ovaries</td>
<td>No (normal renin activity)</td>
<td>Y254D?</td>
<td>No detectable activity</td>
<td>No</td>
<td>557, 612–615</td>
</tr>
<tr>
<td>43 Japanese Sequence variants 56 46 XY</td>
<td>Japanese</td>
<td>Sequence variants</td>
<td>XY</td>
<td>Pigmentation, perineal hypospadias</td>
<td>Yes</td>
<td>Y308X?</td>
<td>No predicted activity</td>
<td>N/A</td>
<td>598</td>
</tr>
<tr>
<td>44 American 57 46 XX 15.6 yr</td>
<td>American</td>
<td>15.6 yr</td>
<td>XX</td>
<td>Hirsutism and/or oligomenorrhea</td>
<td>No</td>
<td>A167V</td>
<td>81.45 ± 0.03%</td>
<td>No</td>
<td>59, 585</td>
</tr>
<tr>
<td>45 American 58 46 XX 7 yr</td>
<td>American</td>
<td>7 yr</td>
<td>XX</td>
<td>Premature pubarche at 4 yr, growth acceleration</td>
<td>No</td>
<td>S213G</td>
<td>58.4 ± 0.8%</td>
<td>No</td>
<td>59, 586</td>
</tr>
<tr>
<td>46 American 59 46 XX 9 yr</td>
<td>American</td>
<td>9 yr</td>
<td>XX</td>
<td>Premature pubic hair</td>
<td>No</td>
<td>L236S</td>
<td>100%</td>
<td>No</td>
<td>59, 585</td>
</tr>
<tr>
<td>47 American 60 46 XX 17 yr</td>
<td>American</td>
<td>17 yr</td>
<td>XX</td>
<td>Hirsutism and/or oligomenorrhea</td>
<td>No</td>
<td>L236S</td>
<td>100%</td>
<td>No</td>
<td>59, 585</td>
</tr>
</tbody>
</table>

The ethnic origin, phenotypic characteristics, and clinical features of the patients are indicated as well as a summary of the functional consequence of mutations or sequence variants in the HSD3B2 gene. The mutations previously designated 186insC/187, 266delA, 273delAA, and 318delC have been changed to 558insC, 818delAA, 797delA, and 853delC, respectively, to follow the current international nomenclature, where the first nucleotide used to assign the name of the mutant is the A of the ATG codon. The apparent activity of mutant enzymes in intact cells was calculated after a 1-h incubation. The values indicated are the mean ± SEM of two independent experiments performed in triplicate. N/A, Not available; ?, undetermined.
diagnosis of this disorder now appears to be a plasma level of 17OH-PREG greater than 100 nmol/liter after stimulation with ACTH. However, it is well recognized that plasma levels of 17OH-PREG and Δ1-DIONE and other Δ4-steroids are frequently elevated in 3β-HSD-deficient patients (13, 56–58, 539, 543–547, 550, 551, 558–560). The T response to hCG is usually poor in infancy (553) but may be substantial in pubertal boys (564). Such observations are consistent with a functional type I 3β-HSD enzyme that is expressed in peripheral tissues. However, the peripheral type I 3β-HSD activity could explain why certain patients were initially misdiagnosed as suffering from 21-hydroxylase deficiency, in view of the elevated levels of 17OH-PREG and mild virilization seen in girls at birth (544). Nevertheless, the significant accumulation of precursors, for instance 17OH-PREG, may also interfere with the RIAs used in routine laboratories to measure levels of 17OH-PREG, although in practice this elevated level of 17OH-PREG is considered beneficial. It has been suggested by Morel et al. (544) that an increased level of 17OH-PREG observed when screening neonates for 21-hydroxylase deficiency should help in making the correct diagnosis of 3β-HSD deficiency. Therefore, measurement of the levels of 17OH-PREG should be performed when an elevated level of 17OH-PREG has been observed in a female neonate without ambiguity of external genitalia or if the patient is a male pseudohermaphrodite (544).

In the presence of premature pubarche with high DHEA-S levels in girls, the diagnosis of attenuated or late-onset 3β-HSD deficiency was often evoked. The late-onset form of 3β-HSD deficiency has been described in older females with hyperandrogenism beginning at adulthood and in children with premature pubarche (565–569). No mutation was found in the HSD3B1 and/or HSD3B2 genes in these patients (569–572), and upon reexamination certain patients no longer showed an elevated Δ4/Δ1 ratio (569, 572).

C. Molecular diagnosis

To date, a total of 37 mutations (including five frameshift, four nonsense, one in-frame deletion, one splicing, and 26 missense mutations) have been identified in the HSD3B2 gene in 60 individuals from 47 families suffering from classical 3β-HSD deficiency as shown in Table 2. In almost all the cases, the characterization of HSD3B2 mutations has provided a molecular explanation for the heterogeneous clinical presentation of this disorder (59). As described above, the high production rate of PROG by the placenta, due to type I 3β-HSD activity is essential for the maintenance of human pregnancy. Consequently, it was suggested that in a fetus homozygous for an HSD3B1 gene defect, the absence of the activity of the placental enzyme would lead to interruption of pregnancy before the end of the first trimester (56–58). The recent data presented by Moisan et al. (59) supports the notion that it is more appropriate to assess the enzymatic activity of transiently expressed mutant proteins using intact HEK293 cells rather than homogenates from cells, because addition of exogenous cofactor can drive a reaction that may not occur in vivo (Fig. 17). For example, enzyme activity can be detected for mutants G15D, L108W, and P186L when assessed in cell homogenates, using PREG as the substrate, whereas no activity can be detected in intact transfected cells using DHEA as the substrate (548). Moreover, this study provides biochemical evidence supporting the involvement of a new molecular mechanism in classical 3βHSD deficiency involving protein instability (Fig. 18). Although the exact molecular and cellular explanation for the apparent instability of various mutant recombinant 3β-HSD proteins in intact transfected HEK293 cells remains to be elucidated, our recent results illustrate that it might be difficult, if not impossible, to rigorously measure the levels of expression of some of these mutant proteins to obtain an accurate estimate of their Vmax value. We are thus suggesting that the varying degrees of protein instability may explain, at least in part, not only the observed decrease in the Vmax values for several mutant proteins and more specifically for those with the L173R or G294V substitution, but also the absence of activity observed in HEK293 cells transiently expressing mutant recombinant proteins A10E, G15D, L108W, P186L, A245P, Y253N, T259M, and T259R.

D. Genotype-phenotype relationships

The current knowledge on the molecular basis of CAH due to 3β-HSD deficiency is in agreement with the prediction that no functional type II 3β-HSD isoenzyme is expressed in the adrenals and gonads of the patients suffering from a severe salt-wasting form (Table 2 and Fig. 17). Furthermore, the missense mutations associated with the severe salt-wasting form of 3β-HSD deficiency (Fig. 17) result in mutant 3β-HSD proteins that retain no detectable enzyme activity, e.g., A10E, G15D, L108W, E142K, P186L, L205P, P222Q, T259R (Table 2). Taken together, all of these results are in perfect agreement with the severity of this form of CAH.

In addition, the current data demonstrates that the non-salt-losing form of classical 3β-HSD deficiency also results from missense mutation(s) in the HSD3B2 gene, which causes an incomplete loss in enzyme activity, thus leaving sufficient enzymatic activity to prevent salt wasting (57–59, 546, 573–575). Indeed, for example, the hormonal profile of two of the individuals bearing the N100S mutation (patients 11, 36, and 37) suggests that salt loss was compensated for by a limited capacity of aldosterone biosynthesis at the price of high renin synthesis (Table 2) (575).

In general, the present functional and biochemical data (Table 2 and Fig. 17) are in close agreement with the severity of the disease in patients suffering from the non-salt-wasting form of 3β-HSD deficiency (576). On the other hand, knowing that all heterozygote carriers of a deleterious mutation in the HSD3B2 gene are asymptomatic, it was unexpected to observe such a relatively high activity of L173R, i.e., 52.8% (Table 2), although the apparent instability of the L173R mutant protein would most likely be involved in further reducing the activity catalyzed by the mutant type II 3β-HSD protein in the cells of the adrenals and gonads in patients 15 and 16. It is also possible that the apparent instability of A245P (patient 22) and G294V (patients 47 and 48) will play a role in further decreasing the activity in these patients, as also suggested by their Vmax/Km values.

Although the results described above illustrate the almost perfect genotype-phenotype relationship associated with
this disorder, there are several examples supporting the notion that there are exceptions to every rule. One of these exceptions is the homozygous A82T mutation that was previously reported in Brazilian patients 7, 8, 9, and 10 (559, 577). In family 7, the homozygous A82T mutation was associated with precocious puberty, whereas in an unrelated family,
family 6, it was found to be associated with male pseudohermaphroditism, although it had no effect in the homozygous female relative. However, it has recently been demonstrated that although the 46XY individual bearing the A10E homozygous mutation, patient 3, presented with the typical phenotype of ambiguous genitalia at birth with normal virilization at puberty, the female patient, patient 2, also harboring this homozygous mutation, presented with spontaneous feminization and menarche (561), which is in contrast to the 46XX patient 14 with severe 3β-HSD deficiency (due to a homozygous W171X mutation) who was hypogonadal (56, 578). These latter findings demonstrate the complex relationships between the genotype and the gonadal phenotype in severe 3β-HSD deficiency and the difficulty in predicting fertility (561). Another good example is the observation that the Brazilian patients 29 and 30 bearing the homozygous mutation T259M suffer from a non-salt-wasting form of the disease (579), whereas the homozygous Taiwanese patient 28 also bearing the T259M mutation and the compound heterozygous French patients 52 and 53 with the heterozygous T259M/867delG mutation, as well as the homozygous Japanese patients 26 and 27 bearing the T259R mutation were all found to be affected by the severe salt-wasting form of the disease. Moreover, the current data strongly indicate instability of the T259M mutant protein (59, 580). Nevertheless, as illustrated in Fig. 17, it was possible to observe a very low residual 3β-HSD activity after a longer incubation period in intact cells expressing the mutant T259M protein when using DHEA as the substrate (59); however, this low activity was more evident when using PREG as substrate (580). It can be speculated that the Brazilian patients possess a different steroid responsiveness, but additional experiments would be needed to better understand this apparent discrepancy.
It was also unexpected to observe that the L236S mutation, which was found in the compound heterozygous non-salt-losing patient 50 (L236S/867delG), possesses the same enzyme activity as the wild-type enzyme with no evidence suggesting that this mutation affects the stability of the protein. Nevertheless, this hypothesis is difficult to reconcile with the well-established fact that heterozygous carriers are asymptomatic (8, 58, 544, 545). On the other hand, it cannot be ruled out that the L236S mutation could be in linkage disequilibrium with another deleterious mutation affecting the expression or the splicing of this gene.

**E. Structure-function relationships**

Finally, the functional characterization of these mutant enzymes also generated valuable information concerning the structure-function relationships of the 3β-HSD superfamily. Indeed, it is of special interest to note that the amino acid residues that are the sites of missense mutations are generally in highly conserved regions in members of the vertebrate 3β-HSD isoenzymes characterized thus far (Fig. 2). This finding strongly suggests the crucial role of these residues for the catalytic activity of these enzymes. It is also of interest to mention that mutations A10E, A10V, and G15D alter amino acid residues in the highly conserved Gly-X-X-Gly region found in all members of the 3β-HSD superfamily (544, 581, 582), which is similar to the common Gly-X-Gly-X-Gly conserved sequence present in most NAD(H)-binding enzymes (68, 583). Furthermore, the striking phenotypic differences observed between the homozygous salt-losing patients 2 and 3, bearing the A10E mutation (561), and the non-salt-losing patients 4 and 5, bearing the A10V mutation, are in accordance with their respective enzymatic properties, as determined in intact cells (Fig. 17). Such a difference may be the result of the observed apparent instability of the A10E protein coupled with the fact that Glu is a negatively charged residue, whereas Val, like Ala, is a nonpolar residue.

Moreover, mutations A82T and G294V create a substituion in each of the two predicted membrane-spanning domains (8, 74). Furthermore, mutation P155L is located in the first of the two characteristic Y-X-X-X-K sequences located in the region from Tyr154 to Lys158 and Tyr269 to Lys273, which is the first of the two characteristic Y-X-X-X-K sequences located in the region from Tyr154 to Lys158 and Tyr269 to Lys273, which is found in the active site of short-chain alcohol dehydrogenases (68, 72, 584). Affinity labeling of purified human type I 3β-HSD identified two tryptic peptides, comprising amino acids Asn176 to Arg266 and Gly251 to Lys274 that should contain residues involved in the putative substrate-binding domain (30). Consequently, the exact role of the first YXXK motif in the 3β-HSD family remains to be confirmed. Finally, recent findings have shown that His261 is a critical amino acid residue for 3β-HSD activity and Tyr253 or Tyr254 participates in the isomerase activity of the human type I 3β-HSD enzyme (23), in addition to providing evidence indicating that Tyr253 functions as the general acid (proton donor) in the isomerase reaction (24). Consequently mutations located within this area will inevitably have a major effect on enzyme activity, as exemplified in the case of the deleterious mutations Y253N, Y254D, T259M, and T259R.

**F. Sequence variants in the HSD3B2 gene vs. nonclassical 3β-HSD deficiency**

Although, as previously mentioned, no mutations have been identified in the HSD3B1 and HSD3B2 genes in patients diagnosed to be suffering from late-onset 3β-HSD deficiency, several sequence variants have been identified in these individuals (A167V, S213G, K216E, and L236S) (585, 586). As illustrated in Fig. 17, in addition to the L236S mutation, the heterozygous A167V sequence variant leads to a protein that has a similar activity to the native enzyme, whereas mutant S213G and K216E proteins have only a minor impact on the activity, retaining 58.4 and 58.95% of the activity of the wild type, respectively (Table 2, patients 57–60) (59). It should also be noted that the mother of patient 57 was also a heterozygous A167V carrier, but did not have any symptoms of hyperandrogenism (585). However, as indicated previously, all reported heterozygous carriers bearing a deleterious mutation in the HSD3B2 gene were typically asymptomatic; these results provide additional molecular proof in support of the conclusion that other genetic or environmental/hormonal influences may contribute to the expression of the observed symptoms (569–572, 587, 588). Thus, the functional data concerning these sequence variants, coupled with the previous studies reporting that no mutations were found in HSD3B1 and/or HSD3B2 genes in the patients affected by premature pubarche or hyperandrogenism (570–572, 587–589), strongly support the conclusion that this disorder does not result from a mutant 3β-HSD isoenzyme. Although the possibility that inherited mutation(s) could be located farther upstream in the putative promoter region of the HSD3B2 gene, leading to an aberrant level of expression of a normal type II 3β-HSD protein cannot be refuted, this hypothesis is markedly weakened by the fact that all patients come from unrelated pedigrees and diverse ethnic origins. In contrast to the 21-hydroxylase deficiency, in which the nonclassical form is an allelic variant of the classical form, the so-called late-onset 3β-HSD deficiency is not an allelic variant of the classical form. On the other hand, because 3β-HSD gene expression and activity are under multiple complex hormonal regulation, it cannot be ruled out that at least some forms of late-onset 3β-HSD deficiency result from a genetic or acquired origin acting indirectly on these modulatory parameters. There is also the possibility of the implication of a steroidogenic enzyme different from known 3β-HSD isoenzymes.

**Acknowledgments**

The authors thank Dr. Robert S. Viger and Dr. Jacques J. Tremblay for helpful discussion and contribution to the manuscript on the role of GATA proteins in 3β-HSO regulation.

Address all correspondence and requests for reprints to: Professor Jacques Simard, Cancer Genomics Laboratory, T3-57, Laval University Medical Center (CHUL) Research Center, 2705 Laurier Boulevard, Québec City, Quebec, Canada G1V 4G2. E-mail: jacques.simard@crchul.ulaval.ca

**References**


60. Bain PA, Meisler MH, Taylor BA, Payne AH 1993 The genes encoding gonadal and nongonadal forms of 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase are closely linked on mouse chromosome 3. Genomics 16:219–223


Abbaszade IG, Clarke TR, Park CH, Payne AH 1995 The mouse 3β-hydroxysteroid dehydrogenase multigene family includes two functionally distinct groups of proteins. Mol Endocrinol 9:1214–1222


Clarke TR, Bain PA, Burmeister M, Payne AH 1996 Isolation and characterization of several members of the murine Hsd3b gene family. DNA Cell Biol 15:387–399


Rainey WE, Bird IM, Mason JI 1994 The NCI-H295 cell line: a
pluripotent model for human adrenocortical studies. Mol Cell Endocrinol 100:45–50


175. Keegan AD, Nelms K, White MJ, Wang LM, Pierce JH, Paul WE 1994 An IL-4 receptor region containing an insulin receptor motif is important for IL-4-mediated IRS-1 phosphorylation and cell growth. Cell 76:811–821


Dhom, G. 1973 The prepuberal and puberal growth of the adrenal (adrenarche). Beltr Pathol. 150:357–377


Doody, K. M., Carr, B. R., Rainey, W., Byrd, W., Murry, B., Strickler, R. C., Thomas, J. L., Mason, J. J. 1990 3β-hydroxysteroid dehydroge-
bovine adrenal cortical cell proliferation by fibroblast growth factor. Lack of effect of epidermal growth factor. Endocrinology 100: 1080–1089


263. Bhattacharya S, Guin S, Bandopadhyay A, Jana NR, Halder S 1996 Thyroid hormone induces the generation of a novel putative...
protein in piscine ovarian follicle that stimulates the conversion of pregnenolone to progesterone. Eur J Endocrinol 134:128–135


267. Rainey WE, Naville D, Cline N, Mason JJ 1991 Prostaglandin E2 is a positive regulator of adenocorticotropin receptor, 3β-hydroxysteroid dehydrogenase, and 17α-hydroxylase expression in bovine adrenocortical cells. Endocrinology 129:1333–1339


298. Picon R, Darmou D, Rouiller V, Duranteau L 1988 Activity of


304. Gonzalez-Robayna IJ, Falender AE, Ochsner S, Firestone GL, Richards JS 2000 Follicle-stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-induced kinase (Sgk): evidence for a kinase-independent signaling by FSH in granulosa cells. Mol Endocrinol 14:1283–1300


336. Hild-Petito S, Stouffer RL, Brenner RM 1988 Immunocytochemical localization of estradiol and progesterone receptors in the mon-
key ovary throughout the menstrual cycle. Endocrinology 123: 2896–2905


344. McAllister JM, Mason JH, Byrd W, Tranl JM, Waterman MR, Endocrinol Metab 71:26–33


375. Tian XC, Berndston AK, Fortune JE 1994 Differentiation of bovine preovulatory follicles during the follicular phase is associated with increases in messenger ribonucleic acid for cytochrome P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase, and P450 17β-hydroxylase, but not P450 aromatase. Endocrinology 136:5102–5110

376. Tian XC, Berndston AK, Fortune JE 1994 Changes in levels of...


3β-hydroxysteroid dehydrogenase activity during human development. J Clin Endocrinol Metab 60:934–939


468. Zawraski G, de Vries JE 1994 Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 15:19–26


478. Labrie F, Belanger A, Cusan L, Candas B 1997 Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. J Clin Endocrinol Metab 82:2403–2409


490. Levy C, Marchut M, Baulieu EE, Robel P 1974 Studies of the...


531. Oh SH, Oh JM, Kim JJ, Choi MK, Park ST, Park OK, Chung YT


Heinrich UE, Bettendorf M, Vecsei P 1993 Male pseudohermaphroditism caused by nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency. J Clin Endocrinol Metab 78:269–278


de Peretti E, Forest MG 1982 Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period. Horm Res 16:10–22


with loss of bile acid synthesis and liver disease. J Clin Endocrinol Metab 88:1833–1841


601. Lafont M 1984 Le déficit en 3β-ol-dehydrogénase chez l’enfant. Lyon, France: Université Claude-Bernard-Lyon 1


Terminology of 3β-HSD
Endocrine Reviews is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.